Synthesis of a New Chiral Polyamine Template – Towards an Active Site Analogue of Vanadium Haloperoxidase

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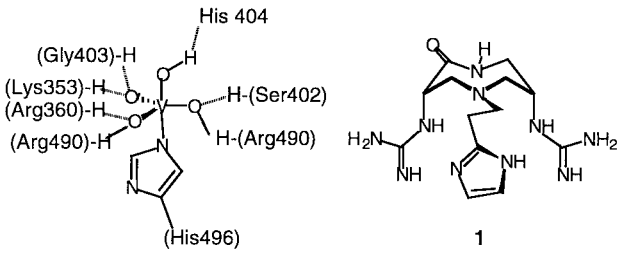
Abstract: The synthesis of a new polyamine template **1** carrying two guanidinium subunits and an imdazole ligand suitable for the recognition of vanadate is described.

Key words: enzymatic halogenation, lactams, polyamines, vanadium haloperoxidases

Vanadium haloperoxidases are found in marine algae and lichens catalyzing the formation of a number of halogenated organic compounds that have biocidal effects and thus often serve as chemical defense of the organism.[1] The X-ray crystal structure of VCPO from the fungus *Curvularia inaequalis* has been recently resolved,[2] revealing a trigonal bipyramidal coordination of V^{5+} (Figure 1). Actually the VO_4^- group is embedded into a network of H-bonds with amino acid residues of the protein, and two histidines are of particular significance:[3,4] His_{496} is coordinating directly to the vanadium in an apical position, whereas His_{404} which is in close proximity to the apical, vanadium–bound oxygen may act as an acid base catalyst.

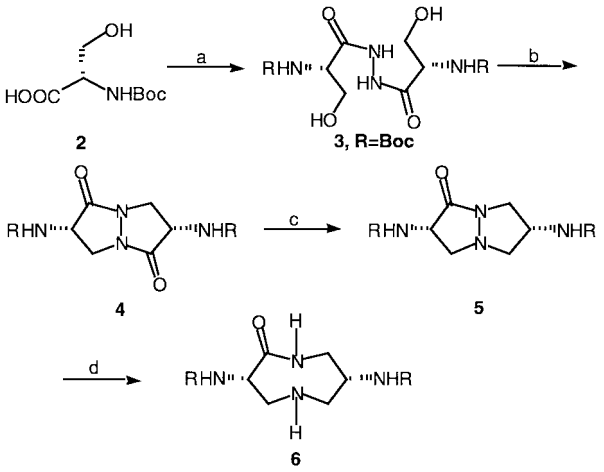
Several functional mimics of vanadium haloperoxidases have been reported.[5] It has been, however, impossible to understand the catalytic cycle of these enzymes completely, i.e. the sequence of events such as binding of hydrogen peroxide, oxidation of chloride, and substrate chlorination, all occuring apparently without changing the oxidation state of vanadium.[6] We wish to report here the synthesis of a template suitable to bind vanadate which was designed according to the spatial arrangement known from the X-ray crystal structure,[2] and taking into account the well established recognition of phosphate, structurally related to vanadate, by bisguanidinium receptors.[7]



Ligand **1** was designed to mimic the structural properties of the active site of vanadiumhaloperoxidase (left).

Figure 1

Molecular modeling[8] revealed that the preferred conformation of the diamino azalactam **1** has both guanidinium subunits in axial positions, facilitating, in the protonated form, the binding of vanadate via hydrogen bridges. The required apical imidazole replacing His_{496} is attached to the secondary ring nitrogen by a short alkyl chain. A convenient route towards **1** proceeds via cyclization of 1,2-diacylhydrazides **3** (easily accessible from **2**) → **4**, followed by selective reduction → **5** and subsequent reductive *N*-*N* bond scission → **6**, (Scheme 1). The key step of this synthesis is the selective monoreduction of the tetrasubstituted diacylhydrazide **4**. This reduction has no precedent but the reported low yield obtained by the reaction of hydrazides with excess of diborane at room temperature.[9]

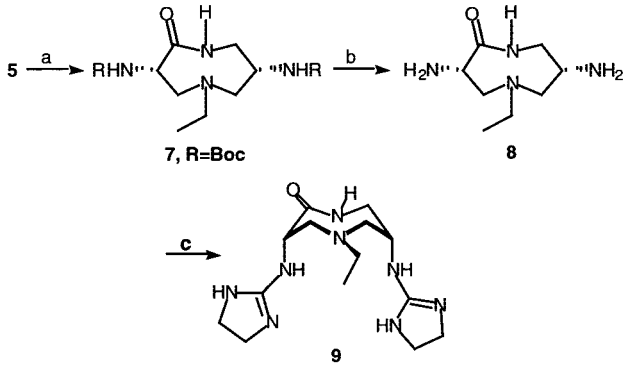


a) 0.5eq NH_2-NH_2, DCC, *N*-hydroxysuccinimide, 65%; b) PPh_3, CCl_4, Et_3N, DMF, 68%; c) BH_3, THF, 27h, Boc_2O, 55%; d) Raney Ni, *i*-PrOH, 92%.

Scheme 1

The preparation of a 1,2-diacylhydrazide from racemic *N*-protected serine has been reported.[10] The coupling of *N*-Boc-L-serine **2** with hydrazine in the presence of DCC and *N*-hydroxysuccinimide[11] occurs without epimerization to yield **3** (Scheme 1). Diazabicyclooctanones have been synthesized by cyclization of 1,2-diacylhydrazides of β-halopropionic acids in the presence of a base.[12] We have prepared the corresponding chloride of **3**[13] which cyclizes in situ under mild conditions to give **4**. The reduction of **4** in the presence of borane (2.2 equivalents) takes place to

give the desired monoreduced product **5**.[14] After quenching of the borane the addition of di-*tert*-butyldicarbonate was required, because the *N*-protecting groups were not completely stable under the reaction conditions. To improve the yield of the monoreduction 14 different reducing agents/reaction conditions were investigated however unsuccessfully. When the reduction of **5** was carried out in EtOH the *N*-ethyl azalactam **7** was obtained.[15,16]

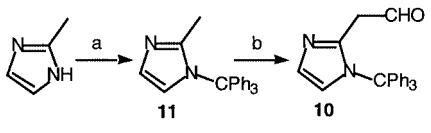


a) Raney Ni, EtOH, 73%; b) HCl•dioxane, THF/CH_3OH (2:1), 97%; c) 4,5-dihydro-1*H*-imidazolium-2-sulfonate, Et_3N, DMF, 77%.

Scheme 2

Deprotection of **7** under standard conditions gave the pure diamine **8**, as the hydrochloride, which can be used directly for the guanidinium functionalization[17,18] to furnish **9** (Scheme 2).

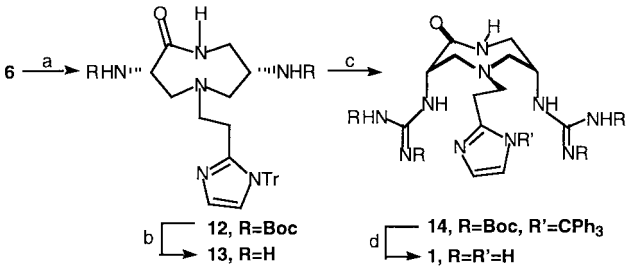
To provide the ethyl-imidazole appendix to the ring nitrogen of the azalactam **6** the imidazolyl aldehyde **10** was prepared using metallation of the *N*-protected-2-methylimidazole **11** under carefully selected conditions to prevent alkylation of C-5[19] (Scheme 3). In principle this can be accomplished by lithium complexation[20] using *N*-ethoxymethyl- or, as shown here, by *N*-trityl protection.[21] Several 1-carbon electrophiles such as CO_2, Eschenmoser's salt,[22] dichloromethane, formic acid,[23] DMF,[24] paraformaldehyde, ethyl chloroformate or methyl cyanoformate[25] were investigated but the most effective electrophile was found to be ethyl formate. The resulting aldehyde **10**[26] is relatively unstable on silica gel and was therefore, without further purification, directly submitted to the Leuckart-Wallach reaction[27] with **6**.



a) $ClCPh_3$, Et_3N, DMF, 95%; b) *n*BuLi, THF, HCOOEt, 5% citric acid aq soln, 80%.

Scheme 3

Condensation of **10** and **6** to the enamine followed by reduction with sodium cyanoborohydride under slightly acidic conditions afforded the fully protected amine **12** in a good yield (Scheme 4). To prevent the cleavage of the trityl group the Boc protecting groups were removed at 0 °C with HCl in dioxane to yield **13** which was used without further purification. For attaching the two required guanidinium groups to **13** we first employed *N,N'*-bis(*tert*-butoxycarbonyl)thiourea, and Mukaiyama's reagent.[28] We found, however, that EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) gave reproducibly better yields of **14**. Deprotection of **14** was carried out under acidic conditions and the resulting polyamine **1** was purified as its picrate which was crystallized several times and finally passed through an ion exchange column to afford pure **1** as the hydrochloride,[29] which was characterized by ^1H NMR (COSY, TOCSY, ROESY), and ^{13}C NMR-spectroscopy.



a) **10**, $CHCl_3$:EtOH (3:1), then $NaBH_3CN$, AcOH, 85%; b) 4 M HCl in dioxane, CH_2Cl_2; c) *N,N'*-Bis(*tert*-butoxycarbonyl)thiourea, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, THF, 44% for b) and c); d) 4M HCl in dioxane, H_2O, 44%.

Scheme 4

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(14) Procedure for the preparation of 3*S*,7*R*-bis-[(*tert*-butoxycarbonyl)amino]-2-keto-1,5-diazabicyclo[3.3.0]octane (**5**): To an ice cooled solution of **4** (1.0g, 2.7 mmol) in dry THF (10mL) a solution of BH_3 in THF (1M, 7.6mL) was slowly added. The resulting mixture was stirred at r.t. for 16h and cooled to 0 °C before the addition of MeOH (4mL) and HCl in dioxane (4M, 2.5mL). After stirring for 7h at r.t. and cooling with an ice bath, a solution of Na_2CO_3 (2M, 2mL) and di-*tert*-butyldicarbonate (1.25g, 5.7mmol) were successively added. To the resulting mixture a solution of Na_2CO_3 (2M, 8.0mL) was slowly added. The resulting suspension was stirred at r.t. overnight, filtered and the filtrate was concentrated. The resulting crude (1.15g) was purified by flash chromatography (70%CH_3CN/1%MeOH/CH_2Cl_2) to afford **4b** (0.575g, 60%): mp 209-210 °C (recrystallization from *i*-PrOH). ^{1}H NMR ($CDCl_3$, ppm, δ):5.97 (br s, 1H, NH), 5.89 (d, *J* = 5.9 Hz, 1H, NH), 4.60 (br s, 1H), 4.52 (dd, *J* = 15.3 and 8.2 Hz, 1H), 3.73-3.64 (m, 2H), 3.39 (m, 1H), 3.20 (br s, 1H), 2.96 (br s, 1H), 2.58 (br s, 1H), 1.39 and 1.38 (2 s, 9 H, *t*-Bu). ^{13}C NMR ($CDCl_3$, ppm, δ):165.6, 155.4, 155.1, 80.2, 79.7, 60.7, 57.7, 54.6, 52.3, 47.0, 28.2. MS (FAB): m/z 356 (M), 301, 245, 158, 57. Anal. Calcd for $C_{16}H_{28}N_4O_5$ (356.43): C, 53.92; H, 7.92; N, 15.72. Found: C, 54.02; H, 7.81; N, 15.75. $[\alpha]^{20}_D$:28° ($CDCl_3$)
(15) Procedure for the preparation of **6** and **7**: To a suspension of **5** (0.44g, 1.25 mmol) in *i*-PrOH (14mL) a slurry of Raney nickel (aprox. 3g, FLUKA 83440, previously washed 2 times with *i*-PrOH) in *i*-PrOH (2mL) was added. The resulting mixture was stirred at 50 °C for 1 h (followed by TLC), cooled and filtered through celite. The crude product was purified by flash chromatography (SiO_2,TBME/Hexanes, 2:1) to give a white solid (**7**, 0.33g, 92%): m. p. 218-220 °C. ^{1}H NMR ($CDCl_3$, ppm, δ):7.28 (br s, 1H, NH), 5.62 (d, *J* = 6.2 Hz, 2H, NH), 4.62 (m, 1H), 3.97 (m, 1H), 3.74 (br s, 1H), 3.29 (dd, *J* = 12.2 and 4.5 Hz, 2H), 2.72 (dd, *J* = 14.4 and 3.7 Hz, 1H), 2.70 (t, *J* = 11.6 Hz, 1H), 2.46 (m, 1H), 1.45 and 1.43 (2 s, 9 H, *t*-Bu). ^{13}C NMR ($CDCl_3$, ppm, δ):176.0, 155.2, 155.1, 79.8, 79.7, 55.0, 52.9, 51.7, 50.8, 43.7, 28.5, 28.3. MS (FAB): m/z 359 (M+1), 303, 247, 57. Compound **7** was prepared according to the same procedure, using ethanol as solvent.
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(18) Procedure for the preparation of 3*S*, 7*R*-bis(4,5-dihydro-1*H*-imidazol-2ylamino)-5-ethyl-2-keto-1,5-diazacyclooctane hydrochloride (**9**): To a solution of **8** (300mg, 1.02mmol) in 5.5mL of DMF, triethylamine (440µl, 3.20mmol) was added under argon. After stirring for 10 min the solution was filtered and 4,5-dihydro-1*H*-imidazole-2-sulfonic acid (290mg,1.94mmol, dissolved in 8mL of DMF) was added over 4h using a syringe pump. The mixture was stirred for 48h at r.t. under argon. The resulting precipitate was collected by filtration and washed with ether. The crude product was adsorbed on a cation exchange column (Sephadex CM C-25, 3 x 10cm) and eluted with a salt gradient (0.1M-0.5M NH_4OAc). The product fractions were collected and lyophilized. The acetate was converted to the chloride by anion exchange (Dowex 1X8, Cl^- form, Fluka, eluent CH_3CN/H_2O 1:1) to afford **9,** (310mg, 77%).^{1}H NMR (H_2O/10% D_2O, ppm, δ):8.21 (d, 1H, *J* = 6.1 Hz), 4.54 (dd, 1H, *J* = 10.5 and 2.9 Hz), 3.76, 3.74 (2s+m, 9H), 3.57 (d, 2H, *J* = 7.16 Hz), 3.26 (dd, 1H, *J* = 14.0 and 6.1 Hz), 3.22 (dd, 1H, *J* = 12.47 and 1.7 Hz), 2.92 (m, 2H), 2.87 (q, 2H, *J* = 7.2 Hz), 1.17 (t, 3H, *J* = 7.2 Hz).^{13}C NMR (D_2O, ppm, δ):171.95, 159.52, 158.54, 54.27, 53.61, 53.51, 52.45, 49.69, 49.60, 43.29, 42.43, 42.39, 9.48. MS (ESI, H_2O/CH_3CN 1:1): m/z 162 $(M+2H)^{2+}$, 323 $(M+H)^+$. $[\alpha]^{20}_D$:-12° (H_2O).
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(26) Procedure for the preparation of 2-(1-tritylimidazolyl)acetaldehyde (**10**):1-Trityl-2-methylimidazole (**11**) was prepared as described in the literature.[21] To a -78 °C cooled solution of 1-trityl-2-methylimidazole (mp 219-221 °C (EtOAc/ hexanes); lit. 217-219 °C) (2g, 6.2mmol) in dry THF (60mL) *n*-BuLi (1.6mol/L, 4mL) was added dropwise. After stirring for 50min at -78 °C, freshly distilled HCO_2Et (2.4mL, 31mmol) was quickly added. The color of the reaction mixture changed from red to pale yellow. After stirring for 15min at -78 °C, citric acid (5%, 20mL) was added. The layers were separated, the organic phase was diluted with EtOAc (50mL), dried (Na_2SO_4), filtered and evaporated. The resulting solid was triturated with hexanes (70mL) and EtOAc (5mL) to give 1.89g of a white solid (**10**, 87%, mp 103-118 °C) of enough quality for the next reaction. ^{1}H NMR (DMSO-d_6, ppm, δ): enol 7.32 (m, Ar, 9H), 7.19 (m, Ar and H-imidazole, 7H), 6.62 (d, J = 1.4 Hz, H-imidazole, 1H), 6.57 (d, J = 6.1 Hz, CHO, 1H), 4.41 (d, J = 6.1 Hz, CH-imidazole, 1H). ^{13}C NMR (DMS*O*-d_6, ppm, δ):151.1, 148.9, 141.7, 129.4, 129.3, 128.5, 128.3, 128.1, 123.2, 119.3, 92.2, 74.7. MS (FAB): m/z 506, 353 (M+1), 243, 165.
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(29) Procedure for the preparation of 3,7-*S*-diguanidino-5-[2'-(imidazolyl)ethyl]-2-keto-1,5-diazacyclooctane hydrochloride (**1**): compounds **6** (400mg, 1.12mmol) and **10** (790mg, 2.23mmol) were dissolved in $CHCl_3$/EtOH 3:1 (10mL). The mixture was stirred for 3h under argon at r.t. $NaBH_3CN$ (207mg, 2.8mmol) was added followed by the slow addition of AcOH (2mL). After 3h the solution was basified with sat. aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layers were dried (Na_2SO_4) and evaporated. Column chromatography (SiO_2, 2% MeOH/CH_2Cl_2) afforded 758mg (95%) of **12**. Compound **12** was dissolved in dry CH_2Cl_2 and 4M HCl in dioxane. After 2h stirring at 0 °C, the solvent was evaporated, the white residue **13** was dried under high vacuum and used without further purification. To a suspension of **13** (1.35g) and di-*tert*-butoxycarbonylthiourea (1.75g, 6.33mmol) in dry THF (21mL) was added Et_3N (2.2mL) and EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1.21g, 6.33mmol). After 3h stirring under argon at r.t., the mixture was washed three times with water, dried ($MgSO_4$) and evaporated. Column chromatography

(SiO_2, hexanes/EtOAc 3:1) afforded 906mg (44% from **12** to **14**) of **14**. Then **14** (188mg, 0.192mmol) was dissolved in 4N aq HCl in dioxane (9mL) and water (1.2mL). After stirring overnight at r.t., the solvent was removed and the residue lyophilized. The white powder obtained was triturated with EtOAc, filtered and dried under vacuum to afford 111mg of impure **1**. It was purified, following a known procedure[30] by conversion to the picrate salt, followed by two recrystallisations from CH_3CN. The picrate salt of **1** was converted to the hydrochloride form by an ion exchange column (Dowex 1X8, Cl^- form, Fluka, eluent THF/H_2O 1:1) to afford 47mg (44%) of pure **1**. ^1H NMR(D_2O, ppm, δ):7.25 (s, 2H, H-C(3‘), H-C(4‘)); 4.58 (m, J = 4.7 and 10.5 Hz, 1H, H-C(3)); 3.76 (d, *J* = 15.5 Hz, 1H, H-C(8)); 3.69 (br s, 1H, H-C(7)); 3.28 (dd, *J* = 2.5 and 15.5 Hz, 1H, H-C(8)); 3.07 (m, 6H, H-C(4), H-C(6), 2H-C(1‘), 2H-C(2‘)); 2.75 (m, *J* = 10.5 Hz, 1H, H-C(4)); 2.66 (br s, 1H, H-C(6)). ^{13}C NMR (D_2O):174.5, 156, 145.3, 118.5, 58, 54, 52, 47, 42.5, 23. FAB-MS: m/z 338 $(M+H)^+$. mp 175-178 °C. $[\alpha]^{20}_D$:+68° (H_2O).

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