and 2.5 equiv of SmI_2) when, after 30 min, excess SmI_2 was eliminated from the reaction mixture by bubbling a stream of air prior to the hydrolysis.

After formation of the alkyl (2*E*,4*E*)-2-alkylalka-2,4-dienoate **4**, the SmI_2-promoted 1,4-reduction of the two conjugated C–C double bonds, which is initiated by oxidative addition of SmI_2, produces an enolate radical **5**,[12] which could explain the observed dimerization at the δ-position of compounds **4a**, and **4e**. A second electron transfer from SmI_2 affords an allylic dianion **6** and its hydrolysis with H_2O or D_2O produces the corresponding compound **2** (Scheme 1).

The observed lower diastereoselectivity in the formation of the C–C double bond in the synthesis of **2a** and **2e**, neither of which contain α-alkyl substituents, may be explained by assuming that in case of the α-alkylated esters **2b**–**d** and **2f**–**i** the s-*cis* conformation of the starting diene is disfavored by steric hindrance produced by the substitution at the α-carbon atom, while the 1,4-reduction of the conjugated C–C double bonds of **2a** and **2e** takes place through both the s-*cis* and s-*trans* conformations. The complete deuteration at C5 instead of at C3 of the dianion **6** could be explained by assuming that in this resonance-stabilized dianion, the 1,4-dianion structure is more stable than the 1,2-dianion structure due to the charge repulsions.

The proposed mechanism is supported by the fact that the synthesis of compounds **2** can be also carried out starting from the corresponding alkyl (2*E*,4*E*)-2-alkylalka-2,4-dienoate **4**. Thus, treatment of ethyl (2*E*,4*E*)-2-hexylhexa-2,4-dienoate (**4d**) with SmI_2 (2.5 equiv) and D_2O (2 mL) for 30 min at room temperature affords **2d** in 84 % yield.

The described methodology can be applied to obtain δ-deuterio-β,γ-unsaturated esters. The reaction of **2h** with LDA, followed by treatment with H_2O afforded ethyl (*E*)-5-deuterio-2-methyl-5-phenylpent-3-enoate (**8h**) with total diastereoselectivity (89 % yield). Taking into account that the C–C double bond of δ-deuterio- and α,δ-dideuterio-β,γ-unsaturated esters can be easily hydrogenated,[13] the proposed methodology can be used to prepare saturated δ-deuterio- and α,δ-dideuterioesters, respectively.

In conclusion, in the presence of D_2O, the simple SmI_2-promoted elimination/reduction sequence provides an efficient method for synthesizing α,δ-dideuterio-β,γ-unsaturated esters **2**, in which the C–C double bond is generated with total or high diastereoselectivity, starting from the readily available α-halo-β-hydroxy-γ,δ-unsaturated esters **1**.

Experimental Section

General procedure: A solution of SmI_2 (2.3 mmol) in THF (24 mL) was slowly added dropwise, under a nitrogen atmosphere, to a stirred solution of **1** (0.4 mmol) in THF (4 mL) at room temperature. After the solution had been stirred for 30 min at the same temperature, H_2O or D_2O (2 mL) were added. The mixture was stirred for 30 min at this temperature and then quenched with aqueous HCl (0.1 M, 5 mL). Usual workup afforded crude β,γ-unsaturated esters **2**, which were purified by column flash chromatography over silica gel (10:1 hexane/ethyl acetate). Yields are given in Table 1.

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Allosteric Regulation of an HIV-1 Protease Inhibitor by Zn^{II} Ions[**]

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Allosteric regulation of protein activity by noncovalent modifiers (ions or neutral molecules) is a feature found in many natural systems including enzymes.[1] The role of these modifiers is to induce conformational changes in the protein that trigger its activity while they are usually not directly involved in the specific function performed by the macromolecule.

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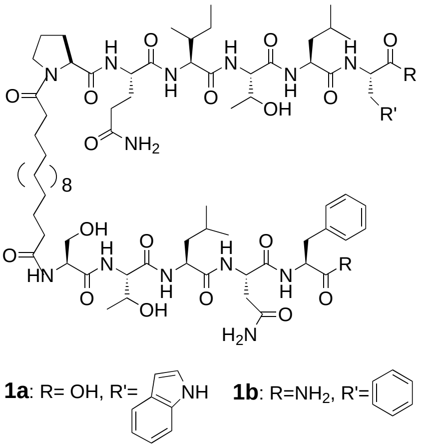
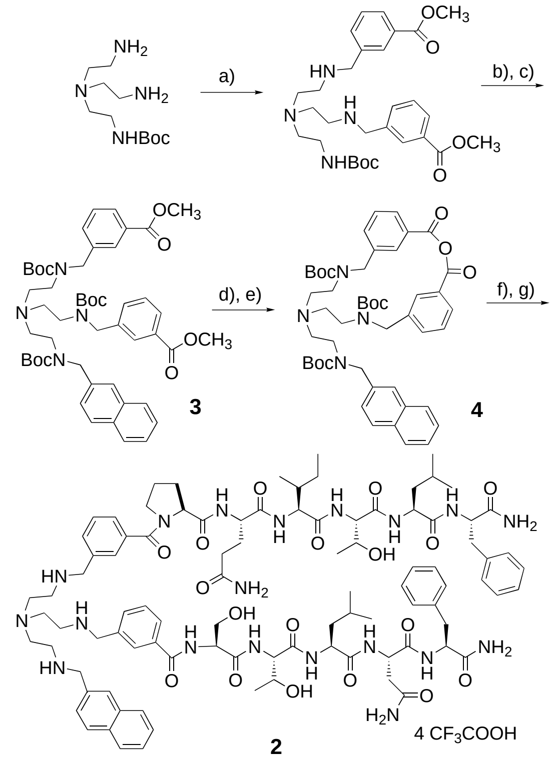
Examples of allosteric control of the properties of artificial systems have also been reported[2] including a very recent example of a phosphodiesterase model[3] whose activity is triggered by remote coordination to metal ions. The latter represents one of the few systems that show allosteric regulation of the activity of a designed synthetic molecular receptor. Herein we describe an example of the control of enzymatic activity by allosteric regulation of the efficiency of a putative inhibitor by Zn^{II} ions.

The enzyme we have studied is HIV-1 protease,[4] a protein whose activity requires the dimerization of two identical subunits. Most of the driving force for the dimerization derives from an interdigitating N- and C-terminal four-stranded, antiparallel β-sheet. HIV-1 protease plays a critical role in viral replication and, consequently, is a key target for the design of inhibitors as potential anti-HIV drugs. Inhibition can result from binding at the active site or, because of the dimeric nature of the protein, from binding at the dimerization interface, which in this case leads to the disruption of the active species, the dimer.[5] The group of Chmielewski,[6] among others, has carried out research in this direction. They have shown that peptides that feature similar sequences as those present at the N and C termini of each monomer of HIV-1 protease and that are cross-linked by a C_{14}-hydrocarbon spacer display significant activity. For instance, **1a** has a remarkable K_i value of 220 nM. Peptide **1b**, in which TrpOH has been replaced with PheNH$_2$, is an even better inhibitor, with a threefold increase in activity.[7] A key aspect of the system is the length of the tether: 14 CH$_2$ units appears to be the most appropriate length for placing the amino termini of the two peptides approximately 10 Å apart, which is the distance found between the N and C termini of each monomer in the enzyme.[8]



We reasoned that the reversible control of the distance between the two tethered peptides in an inhibitor like **1** would lead to the modulation of its activity. For this purpose, we selected tris(2-aminoethyl)amine (TREN) as a metal-binding template to which to cross-link two peptides (analogous to those in **1**) through a rigid aromatic spacer. Molecular modeling predicted that the two N termini of the peptides would be placed close to the 10 Å distance (presumably required for optimum activity) only in the presence of this spacer and when the TREN platform was bound to a metal ion such as Zn^{II}, in accordance with the crystallographic data reported for a similar tetraamine.[2e] On the contrary, the arms of the free ligand, whose nitrogens are protonated at neutral and lower pH values, are forced to stay at a larger distance because of the electrostatic repulsion. For this reasons TREN appeared to be a good candidate as a platform for the control of the conformation of the inhibitor by Zn^{II} complexation, particularly as far as the distance between the two interfacial peptides is concerned. In the design of the system, the third arm, which is necessary to impart rigidity to the complex but is not used for the enzyme recognition process, was considered for the functionalization with a naphthalene unit, to be used as a fluorescent reporter of the protonation and/or Zn^{II} complexation of the TREN subunit. Indeed, molecular sensors for metal as well as for other ions have been reported which are based on this approach.[9]

The synthesis of our target molecule **2** was rather challenging because it required the selective functionalization of the three arms of the tetraamine. Nevertheless **2** was synthesized in a fairly good yield (Scheme 1). Boc-monoprotected TREN



Scheme 1. Synthesis of inhibitor **2**: a) 3-carboxymethylbenzaldehyde, CH$_3$CN, 3 Å molecular sieves, RT, 12 h, then NaBH$_4$, dry CH$_3$OH, N$_2$, RT, overnight, 77%; b) TFA, dry CH$_2$Cl$_2$, then aqueous Na$_2$CO$_3$, 90%; c) 2-naphthaldehyde, CHCl$_3$, 75 °C, 4 h; NaBH$_4$, dry CH$_3$OH, N$_2$, RT overnight, then Boc$_2$O, N$_2$, CH$_2$Cl$_2$, RT overnight, 50%; d) NaOH, CH$_3$CH$_2$OH, 24 h, RT; dilute HCl, 90%; e) DCC, dry CH$_2$Cl$_2$, 20 min, RT, 76%; f) H-Ser(*t*Bu)-Thr(*t*Bu)-Leu-Asn(TrT)-Phe-●, DMF/CH$_2$Cl$_2$, N$_2$, RT, 2.5 h; H-Pro-Gln-Ile-Thr-Leu-PheNH$_2$, HBTU, HOBt, dry DMF, *N*-ethyldiisopropylamine, N$_2$, RT, 24 h; g) TFA (95%), TES (2.5%), H$_2$O (2.5%), 60% from **4**. Boc = *tert*-butoxycarbonyl, TFA = trifluoroacetic acid, DCC = dicyclohexylcarbodiimide, DMF = *N*,*N*-dimethylformamide, HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-1*H*-benzotriazole. ●- represents the Rink resin.

was first converted into the bis(methyl benzoate) derivative. Deprotection, treatment with 2-naphthaldehyde followed by reduction led to the introduction of the fluorescent probe in **3**. Subsequent Boc-protection of the amino groups and hydrolysis of the ester functional groups led to the formation of the key anhydride intermediate **4** upon treatment with DCC. The two peptides, which were prepared by means of conventional solid-phase synthesis on Rink resin, were connected to the platform in two subsequent steps in an excellent overall yield of 60%. After the removal of the resin and of the protecting groups, the putative inhibitor **2** was isolated as the tetra-CF_3COO^- salt.

Although the reported binding constant of TREN to Zn^{II} is rather high ($K_b \sim 10^{15}\,M^{-1}$ for the 1:1 complex),[10] its apparent value is evidently pH dependent because of the competition with the protons. The dependence of the fluorescence intensity of a 50 μM solution of Boc-deprotected ligand **3** on the presence of one equivalent of $Zn(NO_3)_2$ ($\lambda_{ex} = 291$ nm; $\lambda_{em} = 336$ nm) indicates that there is no complex formation below pH 4.0, whereas the complex is fully formed above pH 6.5. The same behavior is expected with **2**. HIV-1 protease is an aspartyl protease and thus shows better activity at slightly acidic pH values (5.0–5.5) than at neutral pH. For this reason we decided to test the inhibitory ability of **2** and its Zn^{II} complex against this enzyme at pH 5.5, at which point approximately 80% of the complex is formed. The inhibition was evaluated by using a fluorogenic substrate assay developed by Toth and Marshall[11] (see Experimental Section for details). The results for **2** and **2**–Zn^{II}, reported in Figure 1 clearly indicate a higher activity of the Zn^{II} complex than that of the free ligand, with apparent IC_{50} values of 6 μM and 28 μM, respectively. The role of Zn^{II} in triggering the enhanced inhibitory activity of the complex is further highlighted in the inset of Figure 1 in which the activity of HIV-1 protease is reported against the [Zn^{II}]:[**2**] ratio. The curve profile indicates that maximum inhibition is attained with the formation of a 1:1 complex, although in agreement with the above-mentioned fluorescence experiments with deprotected **3**, there is not complete inhibition when one equivalent of metal ion is added. Control experiments showed that the efficiency of the Zn^{II} inhibitor is lost by the addition of a slight excess of ethylenediaminetetraacetate (EDTA), which depletes the TREN subunit of the metal ion.[12]

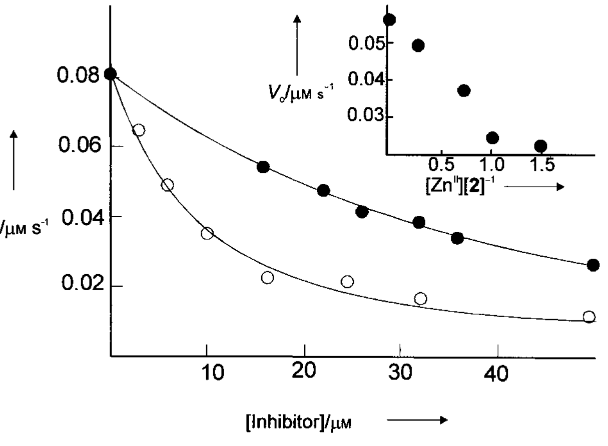


Figure 1. HIV-1 protease activity expressed as initial rate (V_o) of cleavage of the fluorescent substrate Abz-Ile-Nle-Phe(p-NO_2)-Gln-ArgNH_2[11] as a function of the concentration of **2** (●) and its Zn^{II} complex (○). Conditions: [protease] = 25 nM; pH 5.5, 25 °C. Inset: dependence of V_o on the [Zn^{II}]:[**2**] ratio at [**2**] = 16 μM, same conditions as above.

The above results, although interesting, do not provide any evidence of the source of the enhanced potency of inhibitor **2** when it is transformed into its Zn^{II} complex. It has been shown that when the hydrophobic character of the tether is altered by the introduction of an ammonium ion, the activity of an inhibitor such as **1a** is adversely affected.[13] Accordingly, it could well be that a change in the balance between the hydrophobic and hydrophilic character of the TREN platform upon Zn^{II} complexation is at the basis of the observed effect. Thus a Zhang–Poorman plot[14] was used to evaluate if **2** and **2**–Zn^{II} are real competitive inhibitors. Such a plot (Figure 2) reports $E_o/V_o^{1/2}$ against $V_o^{1/2}$ and gives parallel lines in the case of dimerization inhibitors, whereas purely competitive inhibitors are characterized by lines with different slopes that point to a single intercept on the $E_o/V_o^{1/2}$ axis.[5] Our results indicate that **2** and **2**–Zn^{II} are real dimerization inhibitors. The values of K_i for **2**–Zn^{II} and **2** are 1.2 μM and 8 μM, respectively, which shows that Zn^{II} plays a key role in a almost sevenfold enhancement of the activity of **2** upon metal ion complexation. This gain in activity derives from a better binding of the two peptides of the Zn^{II} complex to the dimerization interface of HIV-1 protease. This is in full agreement with molecular modeling studies that indicate a distance between the two peptides in the Zn^{II} complex close to that found between the N and C termini of the native protein.[8] These studies also rule out a possible contribution to the binding[15] by the coordination of the terminal carboxylate of the monomeric protein to Zn^{II}, because of the presence of the rigid aromatic spacer between the peptides and the TREN platform.

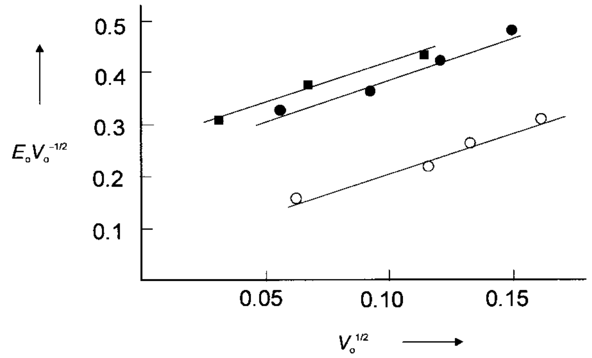


Figure 2. Zhang–Poorman plots[14] for compounds **2**–Zn^{II} (■, 8 μM), **2** (●, 28 μM), and uninhibited protease (○). E_o is the total enzyme concentration; all other conditions as in Figure 1. K_m and k_{cat} for the uninhibited enzyme under the experimental conditions used are 39 μM and 0.77 s^{-1}, respectively.

We note that in spite of the lack of the hydrophobic tether[16] that is present in inhibitors **1**, which contributes to their binding efficiency, **2**–Zn^{II} has a remarkably high activity. The loss of the hydrophobic contribution in **2**–Zn^{II} is likely compensated by a tether between the two peptides which is constrained in a conformation more appropriate for the formation of the interdigitating β-sheet between the metalloinhibitor and the monomeric protein.

In conclusion we have demonstrated that the activity of a dimerization inhibitor of HIV-1 protease derived from cross-linked interfacial peptides can be enhanced by controlling the conformation of their connecting unit through Zn^{II} complexation.[17] This provides a straightforward example of allosteric regulation of the activity of a synthetic inhibitor by a metal ion and shows that although the Zn^{II}-binding process occurs at a remote position, it in fact amplifies the chemical information present in the system (i.e. its ability to recognize a monomer of HIV-1 protease).

Experimental Section

Compound **2** was synthesized according to the route reported in Scheme 1. **4**: ESI-MS (m/z): 837 $[M^+]$. **2**: amino acid analysis: Phe(2) 2.01, Asn(1) 1.1, Leu(2) 2.06, Thr(2) 2.09, Ser(1) 1.03, Gln(1) 0.9, Pro(1) 0.98, Ile(1) 0.87; MALDI-MS (m/z): 1815 $[M+1]$, 1321, 1115; HR-ESI-MS (m/z): 908.056 $[MH_2^{2+}]$. **2**–Zn^{II}: HR-ESI-MS (m/z) 939.013 $[MZn^{2+}]$. (HR = high-resolution, ESI = electrospray-ionization, MALDI = matrix-assisted laser-desorption ionization).

Enzyme assay: Inhibition experiments where performed by using 250 μL of a standard HIV-1 protease solution [50 nM in a buffer prepared with phosphate (20 mM), glycerol (20%), dithiothreitol (DTT; 1 mM) and 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) (0.1%)], which was incubated for 1 h with a solution of the inhibitor with the required concentration (50 μL). The solution was then added to the substrate (Abz-Thr-Ile-Nle-*p*-NO$_2$Phe-Glu-ArgNH$_2$; 200 μL) in the same buffer diluted with dimethyl sulfoxide (DMSO; 10%) to give a substrate concentration of 60 μM. The amount of DMSO was kept constant at 14%. The concentration of HIV-1 protease in the kinetic runs of Figure 1 was 25 nM while in the experiments of Figure 2 it was varied within the 10–80 nM interval. For the determination of K_m and k_{cat}, the substrate was varied within the 10–100 μM interval. Kinetic runs were performed by following the increase of fluorescence emission at 430 nm ($\lambda_{ex} = 360$ nm) for 400 s.

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Regioselective Tetrametalation of Ferrocene in a Single Reaction: Extension of s-Block Inverse Crown Chemistry to the d-Block**

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Duncan M. Tooke

s-Block metal inverse crowns constitute an emerging family of special compounds in which polymetallic amide cationic rings complex anionic guests.[1] They are "inverse" crowns in the sense that their Lewis acidic/Lewis basic sites have been interchanged relative to those in conventional crown ether complexes,[2] that is, here the metal atoms belong to the ring and not to the core. However, the chemistry controlling their formation goes far beyond that of simple macrocyclic host–

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