X-ray Crystal Structure of a Bisubstrate Inhibitor Bound to the Enzyme Catechol-*O*-methyltransferase: A Dramatic Effect of Inhibitor Preorganization on Binding Affinity**

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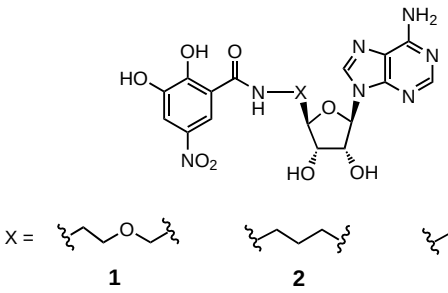
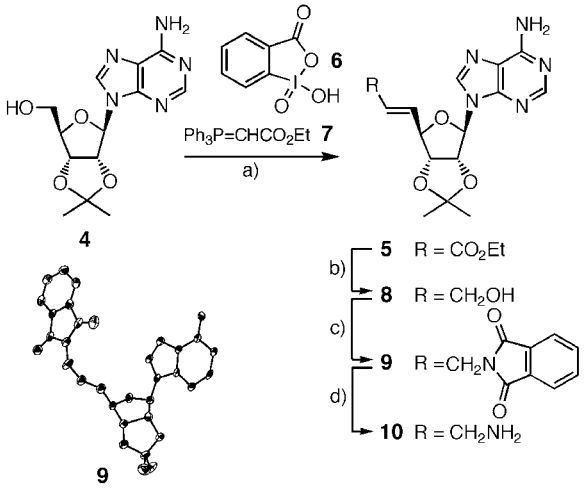
Although many drugs are based on single-substrate analogues, it is well appreciated that enzymes often require, in addition to a substrate, a cofactor for function. The rational design of inhibitors, where a substrate analogue and a cofactor analogue are covalently linked to form a bisubstrate inhibitor, may provide innovative new lead structures in medicinal chemistry that feature both enhanced binding affinity and binding-site selectivity. Some attempts have been made to construct bisubstrate inhibitors for kinases which target both the ATP and the substrate binding sites.[1, 2] Other important targets are methyltransferases that depend on *S*-adenosylmethionine (SAM). The enzyme catechol-*O*-methyltransferase (COMT) catalyzes the methylation of biologically active catechols, such as l-dopa and dopamine, in the presence of SAM and Mg^{2+} ions. The addition of COMT inhibitors to levodopa reduces the extracerebral catabolism of levodopa and increases its elimination half-life, thus ensuring that a higher quantity of orally administered L-dopa reaches its target in the brain.[3] This situation produces a stronger and longer lasting therapeutic effect in patients with Parkinson's disease. Nitro-substituted catechols were found to be potent COMT inhibitors, and two derivatives (tolcapone (Tasmar) and entecapone (Comptan)) have been introduced into the marketplace.[3] Recently, we described the first effective bisubstrate inhibitor **1** for COMT ($IC_{50} = 2\ \mu M$; IC_{50} = concentration of inhibitor at which 50% inhibition of the enzyme is observed) which was developed by rational design using the crystal structure of the quaternary complex between the enzyme, SAM, 3,5-dinitrocatechol, and a Mg^{2+} ion.[4–6] ^1H NMR analysis of a cytosine analogue of **1**[4] in D_2O revealed a strong nuclear Overhauser effect (NOE) between the catechol protons and H-C(5) of the nucleobase and indicates a hydrophobic collapse of the inhibitor.[7] This collapse results in a favorable conformation of the free inhibitor which reduces the binding free energy. Guided by computer modeling studies,[8] we designed compounds **2** and **3** with shorter and conformationally less flexible linkers between the adenine and catechol moieties (Scheme 1). Here,



Scheme 1. Bisubstrate inhibitors **1**–**3** for COMT with different linkers X.

we show that **3** is the most potent bisubstrate inhibitor of COMT introduced thus far and describe the X-ray crystal structure of its ternary complex with COMT and a Mg^{2+} ion.

The synthesis of **2** and **3** started from protected adenosine **4**[9] (Scheme 2).[10] The unsaturated ester **5**[11] was prepared by a convenient one-pot[12] 5′-oxidation with *o*-iodoxybenzoic



Scheme 2. Synthesis of amine **10**: a) Me_2SO, 20 °C, 72 h, 70%; b) DIBAL, CH_2Cl_2, −78 °C, 2 h, 98%; c) phthalimide, DEAD, PPh_3, THF, 20 °C, 2.5 h, 68%; d) $MeNH_2$, EtOH, 20 °C, 16 h, 95%. DIBAL = diisobutylaluminum hydride. DEAD = diethyl azodicarboxylate.

acid (IBX, **6**)[13] and olefination with phosphorane **7**. Reduction with DIBAL gave allylic alcohol **8**, which was transformed into phthalimide **9** by a Mitsunobu reaction.[14] The X-ray crystal structure of **9** (Scheme 2)[15] showed a large spatial separation of the nucleobase and phthalimide moieties, which indicated that the alkene spacer would efficiently prevent the hydrophobic collapse in the free bisubstrate inhibitor **3**. Amine **10** was obtained in excellent yield by the cleavage of phthalimide **9** with methylamine.

Deprotection of **10** gave **11**, which reacted with the activated ester **12** to give target compound **3** (Scheme 3). Catalytic hydrogenation of **10** followed by deprotection afforded **13**, which was coupled to **12** to give the second target compound **2**.

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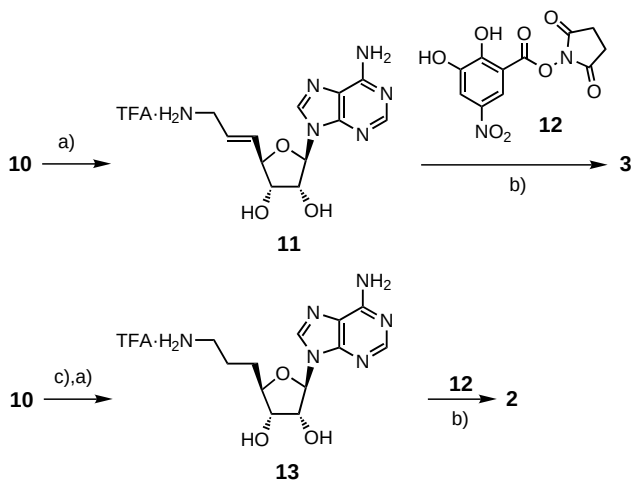
[**] We thank F. Hoffmann–La Roche for generous support of this work. We are grateful to P. Malherbe for the cloning of COMT, P. Caspers for the expression of COMT, A. Cesura for enzyme purification, B. Wipf for fermentation, and H. W. Lahm for sequencing.

  0044-8249/01/11321-4164 $ 17.50+.50/0 *Angew. Chem.* **2001**, *113*, Nr. 21



Scheme 3. Synthesis of bisubstrate inhibitors **2** and **3**: a) CF_3COOH (TFA)/H_2O (5/2), 20 °C, 2 h, 81 %; b) Et_3N, DMF, 20 °C, 24 h, 80 %; c) H_2, Pd/C, EtOH, 20 °C, 2 d, then (a), 74 % (from **10**).

The IC_{50} values, obtained in a radiochemical assay,[4, 16] are listed in Table 1. Compound **2** (IC_{50} 199 nM) has a tenfold higher inhibitory potency than **1** (IC_{50} 2 μM). Removal of the 5′-oxygen atom in **1** produces a shorter, conformationally less-flexible linker which remains sufficiently long to allow the

Table 1. IC_{50} values (uncertainties ±5 %) determined by a radiochemical assay with incubation[4, 16] for the bisubstrate inhibitors **1**–**3** of COMT.

Compound	IC_{50} [nM]
1	2000
2	199
3	9

docking of **2** in both SAM and catechol binding sites. Further rigidification of the spacer by the introduction of a double bond has a tremendous effect on the binding affinity. Compound **3**, which has an IC_{50} value of 9 nM, is the most potent bisubstrate inhibitor for COMT to date. Kinetic analysis[4] shows a competitive inhibition mechanism for **3** with regard to the SAM binding site and a more complex inhibition mechanism with regard to the catechol binding site.

In order to prove our bisubstrate binding model based on computer modeling studies, **3** was co-crystallized with COMT and Mg^{2+} ions. The X-ray structure of the ternary complex formed was then determined at 2.6 Å resolution (Figure 1 a).[17] The inhibitor occupies, as predicted, both the SAM and catechol binding sites. The structure of the ternary complex closely resembles that of the quaternary complex of COMT with SAM, 3,5-dinitrocatechol, and a Mg^{2+} ion—this structure having been used for the rational design of **3**.[4, 5] The protein structure is similar in both complexes, with an overall root mean square (r.m.s.) deviation of 0.4 Å for the Cα atoms. In particular, the intermolecular contacts between the protein and adenosine and catechol moieties are conserved (Figure 1 b). The side chain of Trp 143 is not fully visible in the electron density, that is, its indole group, which shields the adenine and the linker part of the inhibitor from the exterior, is disordered in the ternary complex. The methyl group of

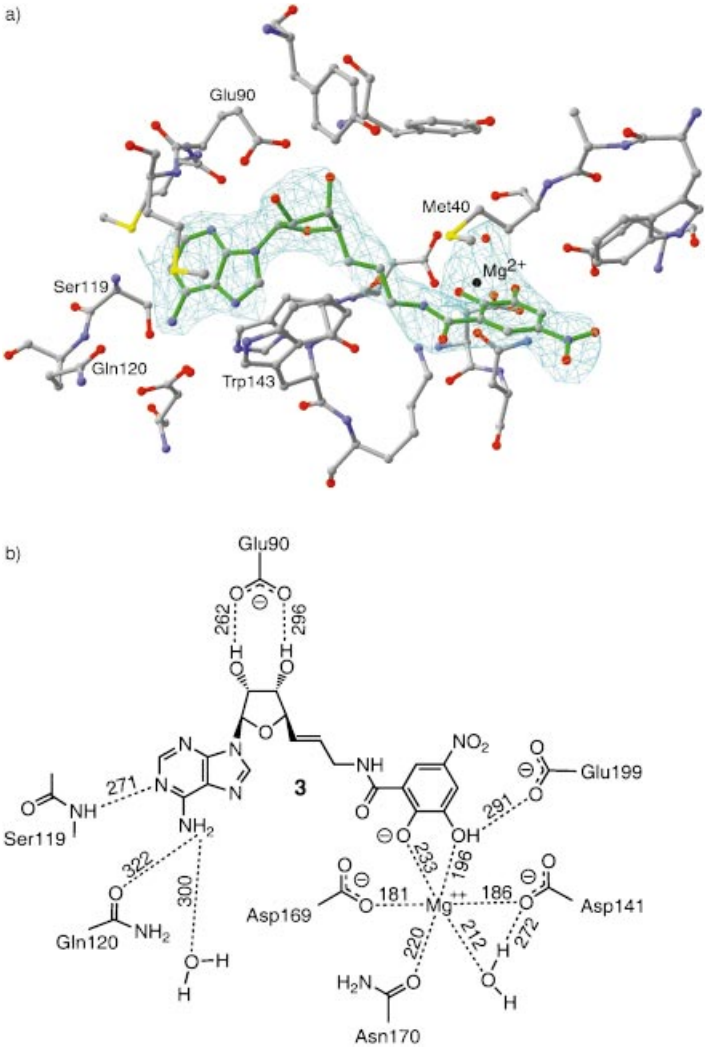


Figure 1. a) The bisubstrate inhibitor binding site in the refined X-ray structure. The $F_{obs} - F_{calcd}$ electron density of **3** is shown contoured at the 2.5 σ level. This electron density was calculated after the first round of refinement when the inhibitor was not yet included into F_{calcd} and is therefore unbiased. Coloring: C: gray, inhibitor framework: green, O: red, N: blue, S: yellow; H atoms omitted. b) Schematic drawing of the H-bonding interactions (dashed lines) in the ternary complex. Distances are given in pm.

Met 40 is rotated by 79° relative to the conformation of Met 40 found in the quarternary complex of COMT, thus making space for the linker of the bisubstrate inhibitor. In the design of next-generation bisubstrate inhibitors of COMT, the flexibility of the side chains of Met 40 and Trp 143 could be taken into account. With the insight gained from X-ray analysis into the molecular recognition principles at the active site of COMT, structural variations of the ribose, catechol, and nucleobase moieties in bisubstrate inhibitors are now targeted.

Received: July 10, 2001 [Z 17469]

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[15] X-ray data for **9**: $C_{23}H_{22}N_6O_5$, $M_r = 462.5$, monoclinic, space group $P2_1$, $\rho_{calcd} = 1.40\ g\,cm^{-3}$, $Z = 2$, $a = 6.933(5)$, $b = 8.12(1)$, $c = 19.48(5)$ Å, $\beta = 91.86(14)^\circ$, $V = 1096(3)$ Å^3, $T = 293$ K. Picker-Stoe diffractometer, $Cu_{K\alpha}$ radiation, $\lambda = 1.5418$ Å. Prismatic crystals (ca. $0.4 \times 0.1 \times 0.015$ mm) were obtained by slow evaporation of a solution of **9** in MeCN. The structure was solved by direct methods (SHELXTL). All heavy atoms were refined anisotropically, H-atoms were fixed isotropically with atomic positions based on stereochemical considerations. Final $R(F) = 0.042$ for 873 reflections with $I > 2\sigma(I)$, $wR(F^2) = 0.11$ for all 1205 data and 308 parameters. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-166783. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
[16] G. Zürcher, M. Da Prada, *J. Neurochem.* **1982**, *38*, 191 – 195. Enzyme preparations were preincubated for 15 min at 37 °C with inhibitor concentrations varying from 10^{-4} to 10^{-9} mol L^{-1}. The reaction was started by adding substrate (1,2-benzenediol, $K_M = 357$ μM[4]) and ^{1}[H]/^{3}[H]SAM ($K_M = 33$ μM[4]), reaching a final substrate concentration of 2.5 mM and a final ^{1}[H]/^{3}[H]SAM concentration of 183 μM. The reaction was stopped after incubating the vials for 15 min at 37 °C by adding acetic acid and 2-methoxyphenol (guaiacol).
[17] Crystals of recombinant rat liver COMT[5] were grown by the vapor diffusion method in space group $P3_221$ with cell axes $a = b = 50.6$, $c = 167.7$ Å. The reservoir solution was 25% polyethylene glycol (PEG) 8000, 100 mM ammonium sulfate, and 100 mM bis-Tris pH 5.5 (Tris = tris(hydroxymethyl)aminomethane). The hanging drop consisted of 1 μL of the reservoir solution and 3 μL of a solution containing 10 mg mL^{-1} COMT, 5 mM $MgCl_2$, and 1.6 mM **3**. A data set to 2.6 Å resolution with an R_{sym} value of 5.4% and a completeness value of 97.8% was collected using $Cu_{K\alpha}$ radiation from a rotating anode source. In the highest resolution shell the R_{sym} value was 27.9% and the completeness value of 95.4%. The starting model for refinement was the protein portion of an isomorphous quarternary COMT inhibitor complex in the crystal (F. K. Winkler, F. Hoffmann – La Roche, Basel, unpublished results, **1994**). The structure was completed using MOLOC[8] and refined with CNX (A. T. Brünger, P. D. Adams, G. M. Clore, W. L. DeLano, P. Gros, R. W. Grosse-Kunstleve, J. S. Jiang, J. Kuszewski, M. Nilges, N. S. Pannu, R. J. Read, L. M. Rice, T. Simonson, G. L. Warren, *Acta Crystallogr. Sect. D* **1998**, *54*, 905 – 921) to an *R* factor of 22.3% and a free *R* factor of 28.0%. The refined model consists of protein residues 3 to 214, a Mg^{2+} ion, 16 water molecules, and **3**. 88.8% of the residues are in the most favored regions of the Ramachandran plot. The atomic coordinates have been deposited to the Brookhaven Protein Databank, with the PDB entry code 1JR4.

A Blue Luminescent Star-Shaped Zn^{II} Complex that Can Detect Benzene**

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Luminescent metal complexes are a fascinating class of molecules that have found applications in many areas of chemistry and materials science. For example, it has been demonstrated that luminescent metal compounds can function as emitters in light-emitting devices.[1] Luminescence is often used as a tool for the detection of a certain weak metal – metal interactions.[2] There are several recent reports on the effect of organic solvents on the luminescence of transition metal compounds,[2] of which most intriguing are the reports by Balch and co-workers that describe an unusual solvoluminescent phenomenon displayed by gold(I) cluster compounds.[2c,d] The high sensitivity of the luminescence of metal complexes to their environment demonstrated in these reports makes such complexes possible fluorescent sensors for specific chemicals. Although there are abundant reports on the use of luminescent transition metal compounds as fluorescent sensors, information on the direct correlation between structures and sensor capability is still scarce. Blue luminescent compounds are a class of highly sought-after materials primarily, because of their potential application as emitters in electroluminescence displays.[3–4] In addition, because most blue emitters have absorption bands in the UV or near-UV region where many aromatic molecules absorb, they have the potential to function as fluorescent sensors for the detection of aromatic molecules, such as benzene, that are an environmental concern.

During our investigation of blue luminescent compounds, we synthesized a new class of blue luminescent organic star-shaped molecules that contain either 7-azaindolyl or 2,2′-dipyridylamino groups. Some of the new star-shaped molecules have been found to be promising blue emitters in electroluminescence devices.[4d, 5] Most interestingly, we found that the new star-shaped molecules are capable of coordinating to various metal centers, which results in the formation of novel coordination compounds. Some of these new compounds are capable of selectively detecting specific small molecules by functioning as fluorescent sensors, an example of which, the Zn^{II} complex of 1,3,5-tris(*p*-(2,2′-dipyridylamino)phenyl)benzene (TPDPB), is described herein.

The novel TPDPB ligand was obtained by a two-step synthesis in an overall 68% yield. The first step is based on a literature procedure[6] and the second step uses Ullmann condensation methods[7] (Scheme 1). The TPDPB ligand displays a broad emission band at $\lambda_{max} = 386$ nm in solution

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[**] This work was supported by the Natural Sciences and Engineering Research Council of Canada and the Xerox Research Foundation.

  0044-8249/01/11321-4166 $ 17.50+.50/0