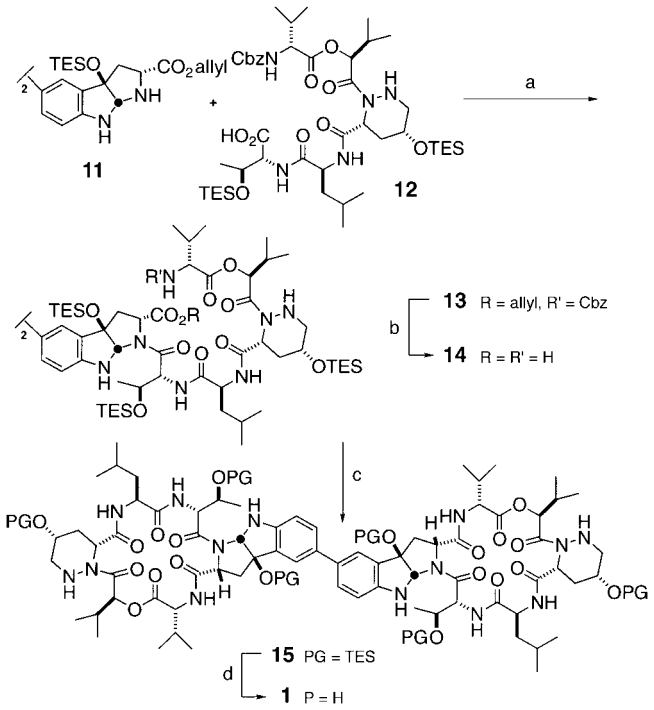
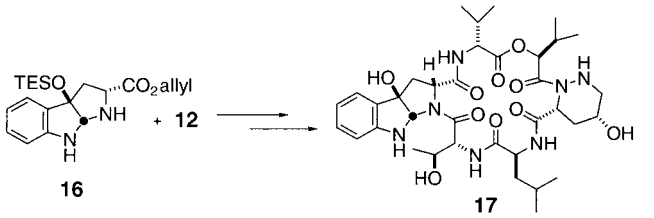
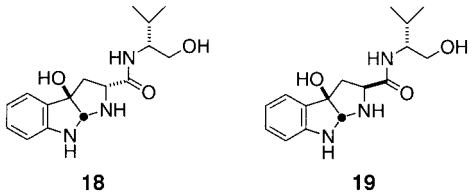


Scheme 3. Synthesis of "*epi-himastatin*" (**1**). a) 1. HATU, HOAt, collidine, CH_2Cl_2, $-10\,^{\circ}C \rightarrow RT$, 65%; 2. TESOTf, 2,6-lutidine, CH_2Cl_2, 63%; b) 1. $[Pd(PPh_3)_4]$, $PhSiH_3$, THF; 2. H_2, Pd/C, EtOAc, 40%; c) HOAt, HATU, $i Pr_2NEt$, DMF; d) TBAF, THF, HOAc, 25% (from **14**). HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethaneaminium hexafluorophosphate, HOAt = 1-hydroxy-7-aza-benzotriazole.



Scheme 4. Synthesis of the "*epi-himastatin*" monomer (**17**).

isopropyl methyl groups of the synthetic *syn-cis* monomer **19** ($\delta = 0.92$ and 0.88)[7] were in much closer harmony with the data ($\delta = 0.94$ and 0.89)[1c] of the degradation product of himastatin (hitherto formulated as **2**) than were those of the synthetic *anti-cis* structure **18** ($\delta = 0.78$ and 0.62).



Therefore, we postulated that the relationship of the *cis* junction and the C_2-carboxamido group in naturally occurring himastatin is *syn* rather than *anti*, as previously proposed. Given the chiroptical data accumulated in the Bristol Myers Squibbs investigation,[1c] we further favored a revision from a D-tryptophan matrix to the L-tryptophan series. Thus, the absolute stereochemistry of the pyrrolindoline junction remains unchanged from the previous assignment. However, the stereochemical assignment of the tricyclic carboxamido centers are reversed (*S*,*S* rather than *R*,*R*). The structure of himastatin is thus assigned as **20**, and that of its valinol degradation product as **21**. In the newly proposed structure of himastatin, the components in the depsipeptide domain are presented in alternating D and L configurations.[10] These various proposals were validated when a total synthesis of himastatin was realized.[11]

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[1] a) K. S. Lam, G. A. Hesler, J. M. Mattel, S. W. Mamber, S. Forenza, *J. Antibiot.* **1990**, *43*, 956; b) J. E. Leet, D. R. Schroeder, B. S. Krishnan, J. A. Matson, *J. Antibiot.* **1990**, 961; c) J. E. Leet, D. R. Schroeder, J. Golik, J. A. Matson, T. W. Doyle, K. S. Lam, S. E. Hill, M. S. Lee, J. L. Whitney, B. S. Krishnan, *J. Antibiot.* **1996**, *49*, 299.

[2] M. Ohno, T. F. Spande, B. Witkop, *J. Am. Chem. Soc.* **1968**, *90*, 6521.

[3] Prepared from D-tryptophan: 1) $anthSO_2Cl$, TEA, THF, H_2O; 2) *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea, CH_2Cl_2 (65% yield overall).

[4] a) The anthracene sulfonamide was readily cleaved with Al(Hg) at room temperature. b) The use of the *tert*-butyl ester afforded a much higher *anti*:*syn* stereoselectivity (>15:1) than the corresponding methyl ester (3:1).

[5] M. Nakagawa, S. Kato, S. Kataoka, S. Kodato, H. Watanabe, H. Okajima, T. Hino, B. Witkop, *Chem. Pharm. Bull.* **1981**, *29*, 1013.

[6] J. K. Stille, *Angew. Chem.* **1986**, *98*, 504; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508.

[7] Compound **12** was synthesized similarly to **20**, which is described in ref. [11].

[8] J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243.

[9] The ^1H NMR spectra of synthetic **1** and **17** are virtually identical upfield of 6.0 ppm.

[10] See M. R. Ghadiri, R. J. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, *366*, 324.

[11] T. M. Kamenecka, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 3166–3168; *Angew. Chem. Int. Ed.* **1998**, *37*, 2995–2998.

Total Synthesis of Himastatin: Confirmation of the Revised Stereostructure**

Theodore M. Kamenecka and Samuel J. Danishefsky*

Dedicated to Professor E. J. Corey

In the preceding communication[1] we have provided the background of the himastatin problem and the findings that necessitated a revision in the assignment of the configuration

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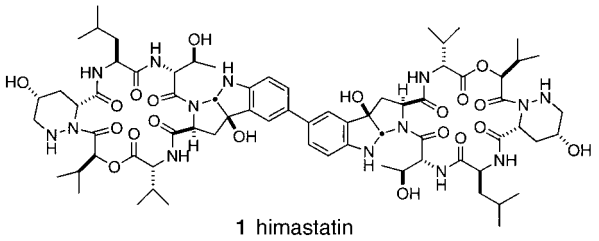
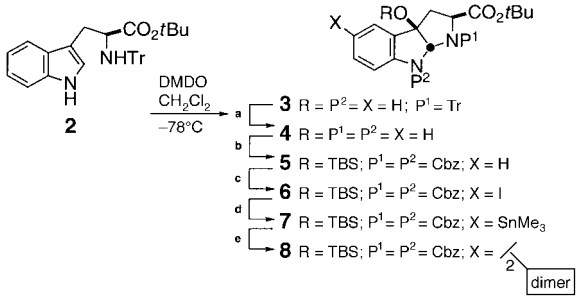
at the tryptophyl carboxyl stereogenic centers (C_2) in each of its two pyrroloindoline domains (see revised structure **1**). Here we describe the total synthesis of himastatin and confirm the structural arguments.



1 himastatin

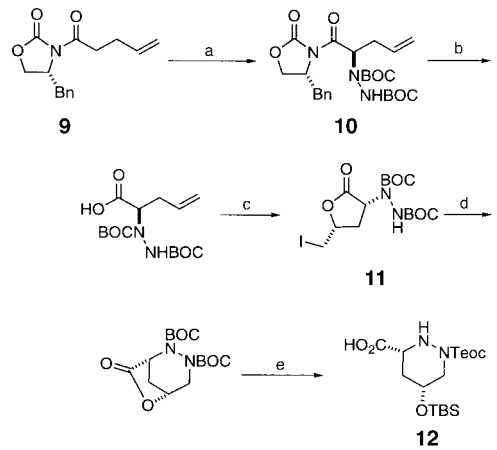
We first turned to the very important matter of gaining stereospecific access to the *syn-cis*-pyrrolo[2,3-*b*]indoline series.[2] Many tryptophan-derived congeners were screened as to their amenability for stereospecific oxidative cyclization in the desired mode. The *t*-butyl ester of N_b-trityl-L-tryptophan (**2**)[3] gave, upon oxidation with 2,2 dimethyldioxirane, **3** in 70% yield, apparently unaccompanied by the *anti-cis* diastereomer (Scheme 1). Following cleavage of the trityl



Scheme 1. Synthesis of **8**. a) HOAc, MeOH, CH_2Cl_2; b) 1. CbzCl, pyridine, CH_2Cl_2; 2. TBSCl, DBU, MeCN; c) ICl, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, 75%; d) Me_6Sn_2, $[Pd(PPh_3)_4]$, THF, 86%; e) **6**, $[Pd_2dba_3]$, $AsPh_3$, DMF, 45°C, 79%. Cbz = benzyloxycarbonyl, dba = dibenzylideneacetone, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMDO = 2,2-dimethyldioxirane, TBS = *tert*-butyldimethylsilyl, Tr = trityl = triphenylmethyl.

group, the two amino groups of **4** were protected as benzyloxycarbamate (N-Cbz) groups (→**5**). Upon reaction with ICl, an iodine atom was introduced at C_5 (→**6**). Part of iodo compound **6** was converted into arylstannane **7**. At this stage, we could take advantage of our ability (as demonstrated for the *anti-cis* series)[1] to join sophisticated pyrroloindolines through carbon–carbon bond formation by an extension of the Stille reaction. Under these conditions, **6** and **7** were coupled smoothly to produce **8**.

A stereoselective route to the 5-hydroxypiperazic acid started with the pentenoic acid derivative **9**.[4–6] Its sodium enolate reacted with bis(*tert*-butyl) azodicarboxylate to produce, stereospecifically, **10** (Scheme 2) Cleavage of the acyl–oxazolidinone bond was accomplished through the agency of lithium hydroperoxide in THF. Iodolactonization of the resultant acid under the influence of titanium isopropoxide gave the desired 2,4-*cis* compound **11** along with its *trans* counterpart in a 6:1 ratio. The iodine functionality was displaced upon deprotonation of the terminal Boc-protected



Scheme 2. Synthesis of **12**. a) NaHMDS, THF, −78°C, Boc–N=N–Boc, 80%; b) LiOOH, THF, 89%; c) NIS, $Ti(OiPr)_4$, 80%; d) NaH, DMF, 74%; e) 1. TFA, MeOH; 2. TeocCl, pyridine; 3. TBSOTf, 2,6-lutidine; 4. LiOH, 82%. Bn = benzyl, Boc = *tert*-butoxycarbonyl, HMDS = hexamethyldisilazane, NIS = *N*-iodosuccinimide, Teoc = 2-(trimethylsilyl)ethyloxycarbonyl, Tf = trifluoromethylsulfonyl, TFA = trifluoroacetic acid.

amino group, and an intramolecular N-alkylation took place to form a bicyclic intermediate. Cleavage of both Boc protecting groups and the lactone, acylation of the remote amino group with TEOCCl (TEOC = 2-(trimethylsilyl)ethyloxycarbonyl), protection of the alcohol functionality with a silyl group, and ester hydrolysis led to **12**.

The other four fragments which were employed in building the peptidal domain were 1) the D-threonine derivative **13** (prepared from D-threonine), 2) FMOC-L-leucine (**14**, commercially available), 3) the hydroxyisovaleryl derivative **15** (prepared from commercially available *S*-hydroxyisovaleric acid), and 4) D-valine, as its TROC derivative **16** (Scheme 3). Coupling of **13** with **14** was followed by cleavage of the FMOC group to release the leucine amino functionality. Compound **17** was acylated with the piperazic acid (**12**) to provide **18**. The relatively unreactive N1 atom of the piperazic acid moiety in **18** was acylated by **15**. Following liberation of the hydroxyl functionality on the isovaleryl group (→**19**), acylation with **16**, and removal of the allyl ester and TEOC protecting groups, the required **20** was in hand.

We next turned to the bis(pyrroloindoline) system **8** (Scheme 4). Significant restructuring of this domain was necessary to render it suitable for initial attachment of the depsipeptide domain (to the pyrroloindoline) as well as for macrocyclization and deprotection. For instance, from parallel studies on a related series, it became clear that we would not be able to remove an angular *tert*-butyldimethylsilyl (TBS) protecting group at C3a when the full macrocycle was in place. Hence, the TBS blocking group was cleaved at the stage of **8** to provide **21**. Moreover, a triethylsilyl (TES) functionality could be introduced at this point through the agency of TESCl in the presence of DBU. The four Cbz functionalities in **22** were discharged, giving rise to **23**. Following protection in the form of FMOC derivative **24**, the *tert*-butyl ester was transformed into the triethylsilyl ester through the agency of triethylsilyl triflate and lutidine. Hydrolysis of this triethylsilyl ester gave rise to **25**, which

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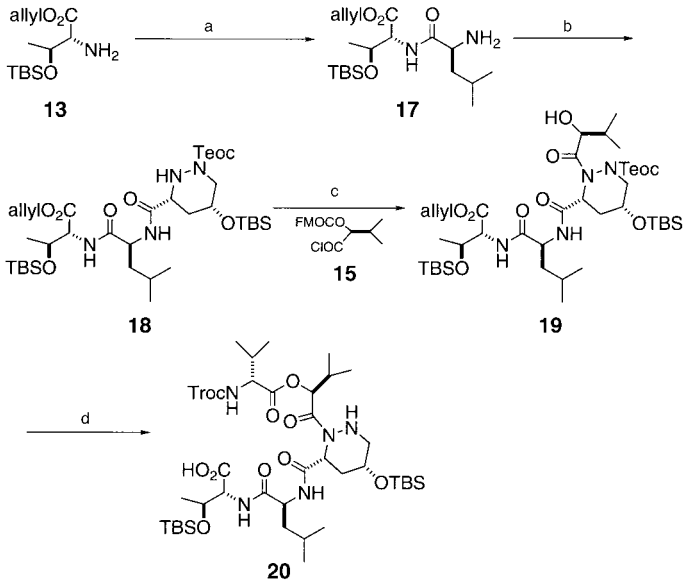
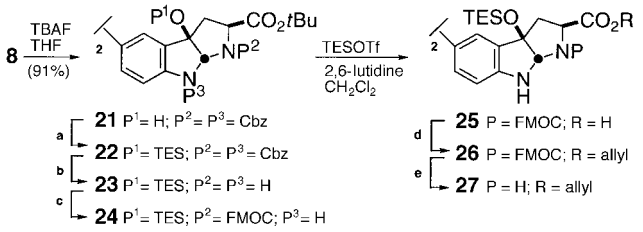


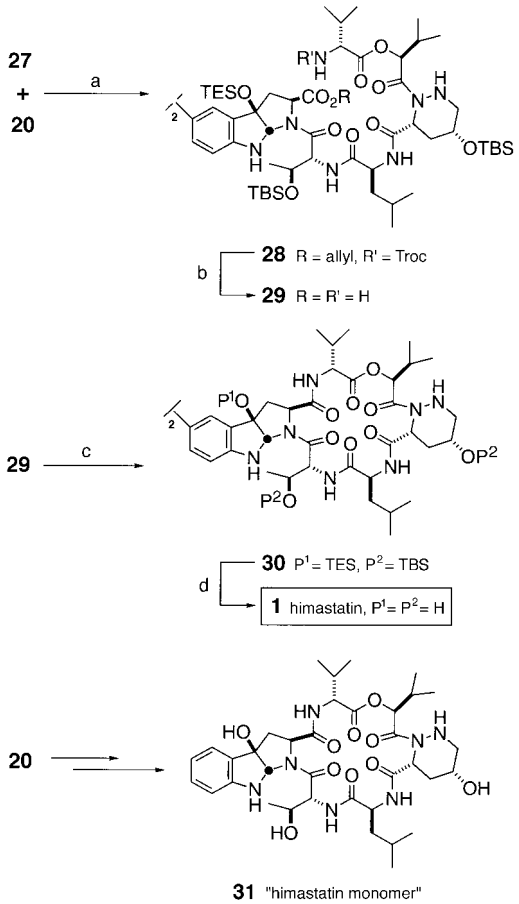
Scheme 3. Synthesis of **20**. a) FMOC-L-leucine (**14**), EDCI, DMAP, CH_2Cl_2; 2. piperidine, CH_3CN, 76%; b) piperazic acid (**12**), HATU, HOAt, collidine, CH_2Cl_2, 95%; c) 1. collidine, CH_2Cl_2, **15**; 2. piperidine, CH_3CN, 96%; d) 1, Troc-D-valine (**16**), IPCC, Et_3N, DMAP, CH_2Cl_2; 2. $ZnCl_2$, CH_3NO_2; 3. TBSOTf, 2,6-lutidine, CH_2Cl_2; 4. $[Pd(PPh_3)_4]$, $PhSiH_3$, THF, 72% (4 steps). DMAP = 4-(dimethylamino)pyridine, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, FMOC = (9*H*-fluoren-9-ylmethoxy)-carbonyl, HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethaneaminium hexafluorophosphate, HOAt = 1-hydroxy-7-aza-benzotriazole, IPCC = isopropenyl chloroformate, Troc = (2,2,2-trichloroethoxy)carbonyl.



Scheme 4. Synthesis of **27**. a) TESCl, DBU, DMF (92%); b) H_2, Pd/C, EtOAc (100%); c) FMOC-HOSu, pyridine, CH_2Cl_2 (95%); d) allyl alcohol, DBAD, PPh_3, THF (90% from **24**); e) piperidine, MeCN (74%). DBAD = di-*tert*-butyl azodicarboxylate, HOSu = *N*-hydroxysuccinimide, TBAF = tetrabutylammonium fluoride, TES = triethylsilyl.

was protected as its allyl ester derivative **26**. Finally, the FMOC functionalities were cleaved with piperidine, thereby providing **27**.

Based on pertinent model studies, we were confident that acylation would occur at N_b of the pyrroloindoline moiety of **27**. Indeed, coupling occurred smoothly at this center with the threonine-derived carboxylic acid **20** to provide **28** in 60% yield (Scheme 5). Following deprotection of the allyl ester and reductive cleavage of the TROC functionality, *seco* structure **29** was in hand. Fortunately, **29** underwent macrolactamization mediated by HATU[7] to give rise to the lactam ester **30**. In the last step of the synthesis the six silyl groups were cleaved to provide himastatin (**1**). The ^1H NMR spectrum of fully synthetic himastatin was identical with that published for the natural product. Furthermore, we independently meas-



Scheme 5. Synthesis of himastatin (**1**). a) HATU, HOAt, collidine, CH_2Cl_2, $-10\,^\circ C \rightarrow$ RT, 60%; b) 1. $[Pd(PPh_3)_4]$, $PhSiH_3$, THF; 2. Pb/Cd, NH_4OAc, THF, 56%; c) HOAt, HATU, iPr_2NEt, DMF; d) TBAF, THF, HOAc, 35% (from **29**).

ured the 500-MHz spectrum of a tiny reference sample of natural himastatin provided by the Bristol Myers Squibb corporation. It was clear by an overlay of the richly detailed spectra that the total synthesis of himastatin had at last been accomplished. This work establishes the correctness of the revised stereostructural formulation of himastatin. With related methodology **31**, containing the full depsipeptide domain on the monomeric parent pyrroloindoline system, has also been synthesized.

As a consequence of the himastatin program, we have learned how to synthesize the pyrroloindoline system in stereospecific form in either the *anti-cis* or *syn-cis* series. We have demonstrated for the first time the applicability of an extended Stille coupling to generate bis(pyrroloindoline) structures in rather delicate and complex settings. The parameters that allow for very subtle protection and deprotection operations of the pyrroloindoline structure have also been established. The biological evaluation of various monomeric and dimeric congeners of himastatin will now proceed in earnest.

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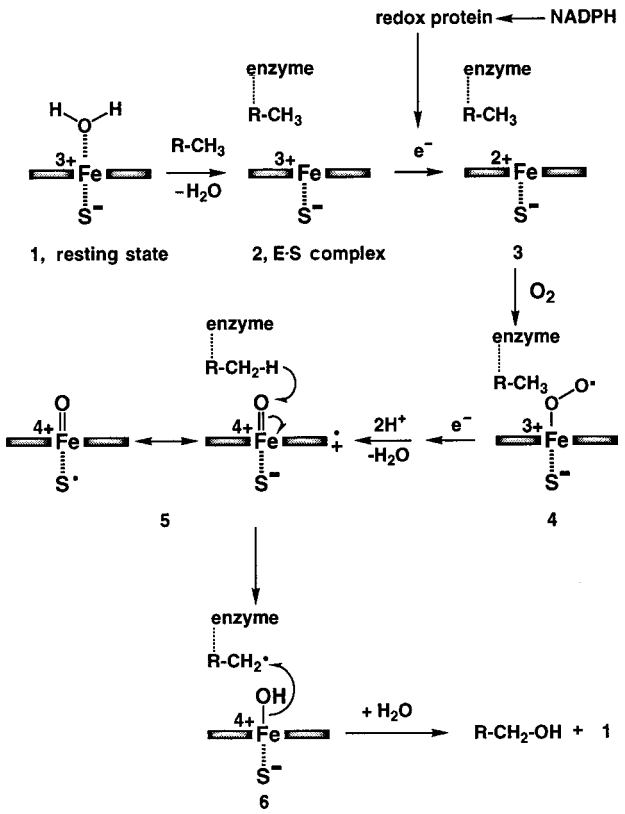
[1] T. M. Kamenecka, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 3164–3166; *Angew. Chem. Int. Ed.* **1998**, *37*, 2993–2995.
[2] See inter alia a) W. E. Savige, *Aust. J. Chem.* **1975**, *28*, 2275; b) M. Nakagawa, S. Kato, S. Kataoka, S. Kodato, H. Watanabe, H. Okajima, T. Hino, B. Witkop, *Chem. Pharm. Bull.* **1981**, *29*, 1013; c) M. Somei, T. Kawasaki, Y. Fukui, F. Yamada, T. Kobayashi, H. Aoyama, D. Shinmyo, *Heterocycles* **1992**, *34*, 1877.
[3] Prepared from L-N_b-trityltryptophan and *N,N'*-diisopropyl-*O-tert*-butylisourea (CH_2Cl_2, RT): P. A. Hipskind, J. J. Howbert, S. Cho, J. S. Cronin, S. L. Fort, F. O. Ginah, G. J. Hansen, B. E. Huff, K. L. Lobb, M. J. Martinelli, A. R. Murray, J. A. Nixon, M. A. Staszak, J. D. Copp, *J. Org. Chem.* **1995**, *60*, 7033.
[4] D. Evans, T. C. Britton, R. L. Dorow, J. F. Dellaria, *J. Am. Chem. Soc.* **1986**, *108*, 6395.
[5] Compound **12** was more conveniently prepared in bulk by a non-stereospecific route starting with (*R*)-methyl 4,5-epoxypentanoate: P. T. Ho, N. Davies, *Synthesis* **1983**, 462.
[6] O. Kitagawa, T. Sato, T. Taguchi, *Chem. Lett.* **1991**, 177.
[7] J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243.

On the Origin of the Low-Spin Character of Cytochrome $P450_{cam}$ in the Resting State—Investigations of Enzyme Models with Pulse EPR and ENDOR Spectroscopy**

Hamed Aissaoui, Rainer Bachmann, Arthur Schweiger, and Wolf-Dietrich Woggon*

The cytochrome P450 enzymes, ubiquitous in nature, are heme–thiolate proteins which are important to the metabolism of endogenous compounds and xenobiotics.[1] The reactivity of these enzymes is associated with an iron(III)–protoporphyrin IX complex in the active site which is bound to the protein through hydrogen bridges of the two propionate side chains and through a thiolate ligand provided by a cysteine residue; the thiolate ligand is coordinated to the iron center from the proximal side of the porphyrin.

Our knowledge of various intermediates in the catalytic cycle of cytochromes P450 (Scheme 1) relies on X-ray structures of different forms of cytochrome $P450_{cam}$[2, 3] and numerous investigations on suitable model compounds.[4] Accordingly, the resting state of cytochrome $P450_{cam}$ (**1**) contains six water molecules in the substrate binding domain (one water molecule is coordinated to the iron center) which are all displaced when the natural substrate camphor is bound. It was recently suggested that Arg299, which according to the X-ray structure forms a salt bridge with one of the heme propionate



Scheme 1. Catalytic cycle of cytochrome P450.

groups, triggers the release of the active-site water cluster through a pathway other than the substrate access channel. On substrate binding, conformational changes of the propionate/Arg299 domain open a channel to a water cluster located close to the protein surface, on the proximal side of the heme.[5]

This change in the ligand sphere of iron is accompanied by a change in the spin-state equilibrium from greater than 96% low-spin Fe^{III} in the resting state **1** to predominantly high-spin Fe^{III} of the E·S complex **2**.[6] The redox potential of the heme–thiolate protein also shifts from −300 mV for **1** to −175 mV for **2**, rendering the latter capable of accepting an electron from NADPH through the redox protein putidaredoxin. Subsequently, the iron(II) complex **3** is formed, which binds oxygen to yield **4**. Reductive scission of the oxygen–oxygen bond furnishes the iron(IV) oxo intermediate **5**, which allows, for example, O insertion into nonactivated C–H bonds (see **6**).[4] Whether O insertion is in general a two-step process, as shown in Scheme 1, or whether it can also proceed in a concerted fashion is currently being disputed.[7]

Ever since the water cluster was discovered in **1**, the origin of the low-spin ground state has been a matter of debate, as it seems unlikely that thiolate and water, both weak ligands, would establish a low-spin iron(III) center. It was therefore suggested that ^-OH rather than water binds to iron or that the hydrogen-bonded water cluster induces a hydroxide-like character at the coordinated water molecule.[2b, 8] From ESEEM studies (ESEEM = electron spin echo envelope modulation) with ^{17}O-enriched water bound to $P450_{cam}$, however, it was concluded that one water molecule binds

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