Tyrosinase Model

DOI: 10.1002/anie.201301249

Catalytic Phenol Hydroxylation with Dioxygen: Extension of the Tyrosinase Mechanism beyond the Protein Matrix**

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A pinnacle of bio-inorganic chemistry is the ability to leverage insights gleaned from metalloenzymes toward the design of small analogues capable of effecting catalytic reactivity outside the context of the natural system.[1,2] Structural mimicry of active sites is an attempt to insert a synthetic catalyst into an enzymatic mechanism. Such a mechanism evolves by selection pressures for efficiency and traverses an energetic path, with barriers and wells neither too high nor too deep in energy—a critical factor of catalytic turnover.[3] An advantage of metalloenzymes over small metal complexes is the site-isolation of the metal center in the protein matrix and the attendant ability to attenuate destructive decay processes—reaction sinks. This protection provides access to thermal regimes that allow barriers and wells to be traversed. Synthetic complexes too must avoid any deleterious reactions, often necessitating the deliberate incorporation of protective superstructures.[4,5] Such limitations make reproducing enzymatic catalytic reactivity in a synthetic complex with native substrates a significant challenge, as evidenced by the dearth of good examples, despite decades of effort.

The intellectual investment of translating a catalytic mechanism from an active site to a synthetic system is justified by a comparison of Nature's dexterity with dioxygen to perform catalytic monooxygenase-type chemistry[6–9] and the dependence of synthetic chemists on exotic reagents for oxygen-atom insertion reactions. Tyrosinase is a ubiquitous dinuclear copper enzyme that catalyzes the hydroxylation of phenols to catechols and the subsequent oxidation of catechols to quinones by activating dioxygen in the form of a side-on-bonded peroxide dicopper(II) species, crystallographically[10] and spectroscopically identified.[11,12] The seemingly simple, regiospecific transformation mediated by tyrosinase is not reproduced easily by synthetic methods, though its importance has been acknowledged by recent efforts to move beyond stoichiometric oxidants,[13] as these sometimes multistep syntheses are often low to moderate yielding and frequently unselective.[14–17] Limited examples of stoichiometric phenolate hydroxylation exist with synthetic, tyrosinase-like side-on peroxide complexes[18–22] formed by oxygenation of copper(I) complexes. Only two complexes achieve significant catalytic phenol hydroxylation using dioxygen: a dinucleating, polydentate imine complex reported by Réglier et al.,[23] and a mononucleating analogue reported by Tuczek et al.[24] both capable of roughly 16 turnovers in the presence of triethylamine. Neither study could identify an operative oxygenated copper species, presumably precluding a more detailed mechanistic analysis of the key substrate hydroxylation step. The possibility of a synthetic side-on peroxide as a catalytic oxidant is suggested by the work of Casella et al. with a dinucleating copper benzimidazole complex, achieving 1.2 turnovers with readily oxidized phenols.[21,25]

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[**] A.H., S.B., A.G., M.R., and S.H.-P. thank the Deutsche Forschungsgemeinschaft (FOR 1405) for financial support. S.H.-P. gratefully acknowledges the Fonds der Chemischen Industrie for a Liebig fellowship, the Bundesministerium für Bildung und Forschung initiative MoSGrid (01IG09006) for computational support, and Prof. Dr. K. Jurkschat for valuable advice. I.I.-B. and O.T. gratefully acknowledge support through the "Solar Technologies Go Hybrid" initiative of the State of Bavaria. Financial support was provided by the U.S. National Institutes of Health (NIH; GM50730) for T.D.P.S. and the William R. and Sara Hart Kimball Stanford Graduate Fellowship for C.C. E.C.W. thanks the CSU Chico College of Natural Sciences for financial support and release time to support this work. Portions of this research were carried out at the Stanford Synchrotron Radiation Laboratory, a national user facility operated by Stanford University on behalf of the U.S. Department of Energy, Office of Basic Energy Sciences.

Supporting information for this article (including materials and methods, synthetic procedures, Raman measurements, XAS spectroscopy details, TD-DFT details, and optimized coordinates of **1**) is available on the WWW under http://dx.doi.org/10.1002/anie.201301249.

Here we report a synthetic catalyst capable of hydroxylating a wide variety of phenols using dioxygen that proceeds through a room-temperature (RT)-stable analogue of oxygenated tyrosinase—a side-on peroxide complex, which possesses ligation and spectroscopic attributes similar to those of the enzymatic active site. Efficient stoichiometric oxidation of phenolates to catecholates at −78 °C is shown, as well as catalytic oxidation of phenols to quinones at room temperature with triethylamine, through a reaction pathway consistent with the generally accepted enzymatic mechanism.[11] Conditions are described in which catalytic turnover is halted and restored at a proposed catecholate–product adduct. This investigation demonstrates that simple structural mimicry suffices to not only transfer the inherent enzymatic catalytic reactivity into a synthetic complex, but that bioinspiration is a viable stategy of pursuing selective transformations of substrates beyond scope of the enzyme.[26]

Oxygenation of [Cu^{I}{bis(3-*tert*-butyl-pyrazolyl)pyridylmethane}]SbF_6 in dichloromethane at −78 °C results in near-quantitative formation (vide infra) of [Cu_2O_2(HC(3-*t*BuPz)$_2$(Py))$_2$](SbF_6)$_2$ (**1**), a side-on peroxide dicopper(II) complex (Figure 1 A), as evidenced by the characteristic O–O stretch in its resonance Raman spectrum at 750 cm^{-1}, which shifts by 39 cm^{-1} upon $^{18}O_2$ substitution (Figure 1 C).[27,28] The expected mass signature and isotope pattern observed in the cryo-ESI-TOF mass spectrum also shifts appropriately upon oxygenation with $^{18}O_2$ (Figure S3 in the Supporting Information). The ligand-to-metal-charge-transfer (LMCT) features at 350 nm (20 $mM^{-1}cm^{-1}$) and 550 nm (1 $mM^{-1}cm^{-1}$) have a 20:1 intensity ratio,[22,27] similar to those of oxy-tyrosinase and oxy-hemocyanin,[29] and the feature near 412 nm (0.9 $mM^{-1}cm^{-1}$) is assigned tentatively to a pyrazole/pyridine $\pi^*\rightarrow d_{xy}$ charge-transfer transition, based on a natural transition orbital analysis of a TD-DFT calculated spectrum (TD-DFT = time-dependent density functional theory; Figure 1 B).[22,30] The DFT-optimized structure of **1** predicts a planar Cu_2O_2 core with a Cu–Cu separation of 3.57 Å, in line with the 3.51 Å distance determined by Cu K-edge extended X-ray absorption fine structure models (Table S2 and Figure S5 in the Supporting Information).[27,28] Taken together, these data fully support the structural homology between tyrosinase and **1**.

The formation of **1** is effectively quantitative (> 95 % yield) in a variety of solvents at −78 °C, as assessed by iodometric titrations of the released peroxide after treatment with trifluoroacetic acid.[22,31] The complex is stable for weeks in CH_2Cl_2 at −78 °C, yet decays within 1 day in tetrahydrofuran or acetone. Compound **1** reacts within 60 min with a wide variety of sodium phenolate salts (5 equiv), both electron-rich and -deficient; the side-on peroxide oxidant is efficiently consumed releasing catechol products after an acidic workup (Table 1 A). Using a 1:1 oxidant/phenolate stoichiometry, impressive catecholate yields (> 90 %) are possible at −78 °C, albeit reaction times of nearly 1 week are required. Expectedly, the mass of the *p*-methoxy-1,2-catechol product is shifted by 2 a.u. if **1** is formed with $^{18}O_2$, indicating dioxygen as the oxygen atom source.[32]

From kinetic data, the hydroxylation of phenolate to catecholate by **1** is best understood as a second-order process: first order in [**1**] and first order in [phenolate], with a pre-equilibrium binding event and a rate-limiting oxidation step, presumably C–O bond formation. An intramolecular competitive kinetic isotope effect of 1.2(2) measured at −78 °C with 2-D-4-*tert*-butylphenolate excludes rate-limiting C–H bond cleavage. The observed rate constants k_{obs} saturate with respect to added phenolate (Figure 2), consistent with an initial phenolate-binding equilibrium K_{eq}, followed by an intramolecular rate-determining oxidation step k_{ox} from a substrate–complex adduct (Table S1 in the Supporting Information),[18,32,33] as electron-deficient phenolates clearly react more slowly. A plot of $\ln(k_{ox})$ versus σ_p^+ for a variety of phenolates gives a Hammett parameter $\rho = -0.99$ (Figure 2), consistent with the trend reported for tyrosinase ($\rho = -1.8$ to -2.2)[34,35] and in line with an electrophilic aromatic substitution mechanism.

At room temperature in CH_2Cl_2, **1** is formed quantitatively but decays irreversibly with a half-life of 30 min. However, this rare stability allows for catalytic hydroxylation of phenols to quinone products at room temperature. Only two other synthetic side-on peroxide species have greater thermal stability, but exogenous substrate reactivity has not been reported.[36,37] With 25 equiv of *p*-methoxyphenol and 50 equiv of triethylamine under 1 atm of O_2,[23,24,38] 10 equiv of quinone is formed in 1 h or 15 equiv in 24 h (Table 1 B), as assessed by the characteristic optical feature of quinone at 400 nm (Figure S1 in the Supporting Information). Slower catalytic reaction rates are observed with more electron-deficient phenols. At these higher temperatures, the oxidation of the catechol to quinone and its subsequent release is proposed to

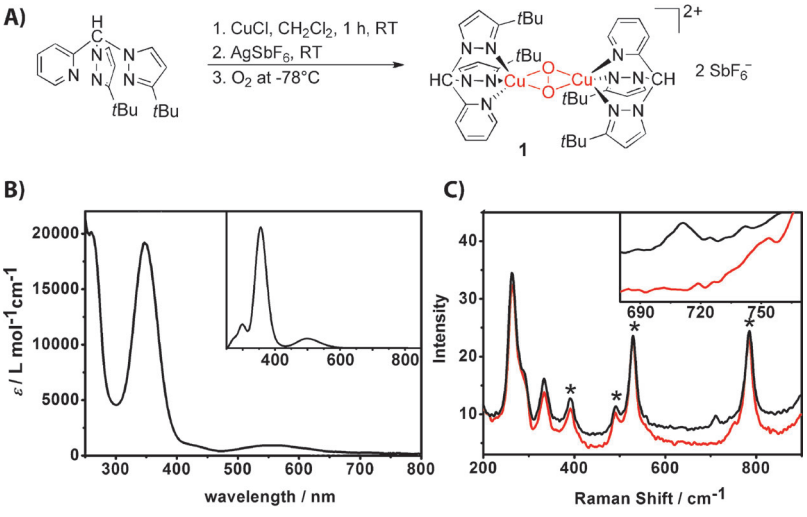
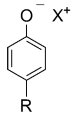


Figure 1. A) Preparation of the side-on peroxide species **1**. B) Absorption spectra of **1**; inset: TD-DFT-predicted optical spectrum of **1**. C) Resonance Raman spectra of 1 in acetone with excitation at 412 nm (red: $^{16}O_2$, black: $^{18}O_2$, asterisks (*): solvent peaks); inset: isotopic shift of the feature at 750 cm^{-1}.

Table 1: A) Reaction of phenolic substrates and **1** under N_2 atmosphere at −78 °C.[a] B) Catalytic oxidation of phenols to quinones.[b]

A)



R	X^+	Equiv[c]	Time	% catechol[d]
OMe	Na^+	5	5 min	>95
Me	Na^+	5	5 min	>95
Cl	Na^+	5	5 min	>95
H	Na^+	5	5 min	>95
F	Na^+	5	5 min	>95
COOMe	Na^+	5	60 min	>95
CN	Na^+	5	60 min	>95
NO_2	Na^+	5	60 min	>90
estrone	Na^+	5	5 min	>90
8-hydroxyquinoline	Na^+	5	5 min	>90
N-acetyltyrosine ethyl ester	Na^+	5	5 min	>90
OMe	$HNEt_3^+$	5	10 min	>95
Me	$HNEt_3^+$	5	10 min	>95
OMe	Na^+	1	7 days	>90
OMe	$HNEt_3^+$	1	10 days	>90
estrone	Na^+	1	8 days	>90
8-hydroxyquinoline	Na^+	1	8 days	>90
N-acetyltyrosine ethyl ester	Na^+	1	5 days	>90

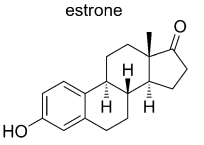
[a] All reactions performed in CH_2Cl_2 at 1 mM [Cu] and quenched with acid at -78°C after the given reaction time. [c] Equiv relative to **1**. [d] Yields based on the concentrations of **1**. No quinone or biphenol products were observed.

B)

Substrate	Time	Turnovers[e]
p-methoxyphenol	1 h	10
N-acetyltyrosine ethyl ester	16 h	15
estrone	6 h	4
8-hydroxyquinoline	16 h	8

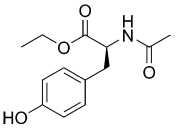
[b] All reactions performed in CH_2Cl_2 at 25°C under 1 atm of O_2 with 25 equiv of substrate, 50 equiv NEt_3 and [Cu] = 1.0 mM. [e] Turnover numbers based on the concentration of **1**.

estrone



8-hydroxyquinoline

N-acetyltyrosine ethyl ester



drive the reduction of the Cu^{II} centers back to a Cu^{I} state, permitting re-oxygenation to **1** (Scheme 1).

Three conditions appear to be necessary for catalytic turnover and support operation through a tyrosinase mechanism: ambient reaction temperatures (25 °C), proton management, and excess dioxygen. Under catalytic reaction conditions at −78 °C, rapid formation of a green species is observed, tentatively assigned as a Cu^{II} catecholate complex (Scheme 1), as one equiv of *p*-methoxy-1,2-catechol is formed as the sole product upon an acidic quench at this temperature. If warmed to 25 °C, this green species yields a quinone rather than a catecholate product, suggesting catecholate-binding inhibition at low temperatures (Scheme 1). Phenol deprotonation and binding to a copper center is essential in the catalytic oxidation process, as exclusion of triethylamine results in the return of unreacted phenol at −78 °C even after a reaction time of hours. After phenol deprotonation, the resultant triethylammonium cation presumably provides the necessary proton for the protonation of hydroxide to water as catecholate reduces Cu^{II} to Cu^{I}.

Beyond the ability to catalytically oxidize a tyrosinase substrate, *N*-acetyltyrosine methyl ester,[39] to its dopaquinone form, the oxidative reactivity of **1** can be extended to more complicated phenols, outside the typical substrate scope of the enzyme (Table 1 B). Estrone, an estrogenic hormone, is regioselectively *ortho*-hydroxylated to 3,4-estrone-*o*-catechol in > 90 % yield at −78 °C in less than 5 min when 5 equiv of its sodium salt are added relative to **1** (Table 1 A). The 3,4-estrone-*o*-quinone is formed under the catalytic conditions at 25 °C with 4 turnovers.[32] While mushroom tyrosinase itself

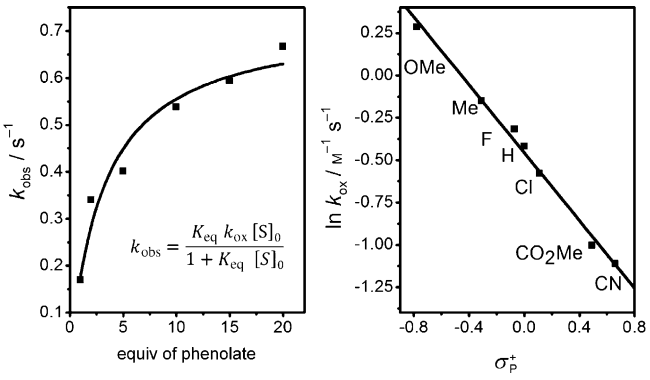
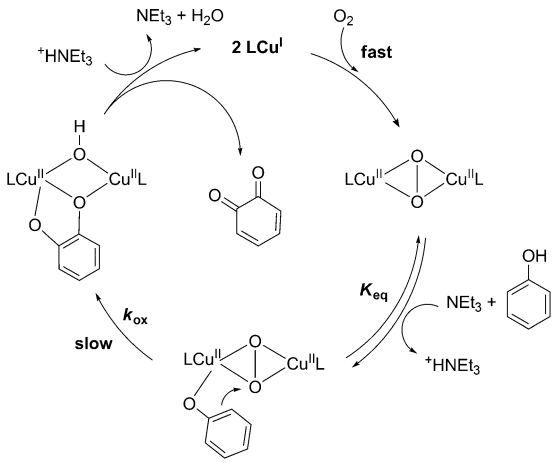


Figure 2. Reactivity of **1** at −78 °C in CH_2Cl_2. Left: Substrate-binding kinetics of the stoichiometric hydroxylation reaction with 4-fluorophenolate. Right: Hammett plot for the stoichiometric hydroxylation reaction with 1–20 equiv of various *p*-substituted phenolates.



Scheme 1. Proposed catalytic mechanism of phenol oxidation by **1** in the presence of triethylamine.

can oxidize estrone,[40] 8-hydroxyquinoline is not a viable substrate,[32] possibly due to the steric demands of the fused ring structure positioned *ortho* to the phenol oxygen (Table 1B).[26] Compound **1** oxidizes 8-hydroxyquinoline both stoichiometrically to the catechol (7,8-dihydroxyquinoline) at −78°C, and catalytically to its quinone at 25°C (quinoline-7,8-dione; 8 turnovers; Table 1B), extending the substrate scope beyond that possible in the enzymatic system.[2] The substrate flexibility of **1** may thus make it a potentially useful reagent for the efficient stoichiometric conversion of a wide variety of phenolates to catechol or a catalyst for the multi-turnover oxidation of phenols by dioxygen to quinones, which are readily reduced back to catechols.[17]

Oxygen-insertion reactions that use dioxygen directly are extremely limited, despite the indisputable advantages to using Earth's ready supply of dioxygen. The difficulty lies in directing the oxidative power of dioxygen and in assuring that reactions profiles do not have insurmountable barriers or thermodynamically overstabilized intermediates. In this work, the essence of the tyrosinase enzyme mechanism, founded on the oxidizing power of dioxygen, is translated from its protein environment into a small complex through only approximate structural mimicry of the oxygenated active site, yet selective and efficient catalytic *ortho*-hydroxylation reactivity results. This strategy of copying pre-existing reactivity from Nature opens the door not only to challenging organic transformations, but for developing useful synthetic tools with substrate scopes beyond those of biological systems.

Received: February 12, 2013
Published online: April 22, 2013

Keywords: bioinorganic chemistry · copper · dioxygen · homogeneous catalysis · tyrosinase

[1] L. Que, Jr., W. B. Tolman, *Nature* **2008**, *455*, 333 – 340.
[2] V. Mahadevan, R. J. M. Gebbink, T. D. P. Stack, *Curr. Opin. Chem. Biol.* **2000**, *4*, 228 – 234.
[3] S. Kozuch, S. Shaik, *Acc. Chem. Res.* **2011**, *44*, 101 – 110.
[4] J. P. Collmann, *Acc. Chem. Res.* **1977**, *10*, 265 – 272.
[5] M. Momenteau, C. A. Reed, *Chem. Rev.* **1994**, *94*, 659 – 698.
[6] S. Itoh, S. Fukuzumi, *Acc. Chem. Res.* **2007**, *40*, 592 – 600.
[7] *Coppper-Oxygen Chemistry* (Eds.: K. D. Karlin, S. Itoh, S. Rokita), Wiley, Hoboken, **2011**.
[8] "Metal-Oxo and Metal-Peroxo Species in Catalytic Oxidations": *Structure and Bonding* (Ed.: B. Meunier), Springer, Berlin, **2000**.
[9] L. Q. Hatcher, K. D. Karlin, *Adv. Inorg. Chem.* **2006**, *58*, 131 – 184.
[10] Y. Matoba, T. Kumagai, A. Yamamoto, H. Yoshitsu, M. Sugiyama, *J. Biol. Chem.* **2006**, *281*, 8981 – 8990.
[11] E. I. Solomon, U. M. Sundaram, T. E. Machonkin, *Chem. Rev.* **1996**, *96*, 2563 – 2605.
[12] M. Rolff, J. Schottenheim, H. Decker, F. Tuczek, *Chem. Soc. Rev.* **2011**, *40*, 4077 – 4098.
[13] C. Huang, N. Ghavtadze, B. Chattopadhyay, V. Gevorgyan, *J. Am. Chem. Soc.* **2011**, *133*, 17630 – 17633.
[14] T. V. Hansen, L. Skattebøl, *Tetrahedron Lett.* **2005**, *46*, 3357 – 3358.
[15] B. Gigante, C. Santos, A. M. Silva, M. J. M. Curto, M. S. J. Nascimento, E. Pinto, M. Pedro, F. Cerqueira, M. M. Pinto, M. P. Duarte, A. Laires, J. Rueff, J. Goncalves, M. I. Pegadod, M. L. Valdeirad, *Bioorg. Med. Chem.* **2003**, *11*, 1631 – 1638.
[16] S. Itoh, M. Ogino, S. Haranou, T. Terasaka, T. Ando, M. Komatsu, Y. Ohshiro, S. Fukuzumi, K. Kano, K. Takagi, T. Ikeda, *J. Am. Chem. Soc.* **1995**, *117*, 1485 – 1493.
[17] A. Pezzella, L. Lista, A. Napolitano, M. d'Ischia, *Tetrahedron Lett.* **2005**, *46*, 3541 – 3544.
[18] S. Itoh, H. Kumei, M. Taki, S. Nagatomo, T. Kitagawa, S. Fukuzumi, *J. Am. Chem. Soc.* **2001**, *123*, 6708 – 6709.
[19] L. Santagostini, M. Gullotti, E. Monzani, L. Casella, R. Dillinger, F. Tuczek, *Chem. Eur. J.* **2000**, *6*, 519 – 522.
[20] L. M. Mirica, M. Vance, D. Jackson Rudd, B. Hedman, K. O. Hodgson, E. I. Solomon, T. D. P. Stack, *J. Am. Chem. Soc.* **2002**, *124*, 9332 – 9333.
[21] S. Palavicini, A. Granata, E. Monzani, L. Casella, *J. Am. Chem. Soc.* **2005**, *127*, 18031 – 18036.
[22] C. Citek, C. T. Lyons, E. C. Wasinger, T. D. P. Stack, *Nat. Chem.* **2012**, *4*, 317 – 322.
[23] M. Réglier, C. Jorand, B. Waegell, *J. Chem. Soc. Chem. Commun.* **1990**, 1752 – 1755.
[24] M. Rolff, J. Schottenheim, G. Peters, F. Tuczek, *Angew. Chem.* **2010**, *122*, 6583 – 6587; *Angew. Chem. Int. Ed.* **2010**, *49*, 6438 – 6442.
[25] L. Casella, M. Gullotti, R. Radaelli, P. Di Gennaro, *J. Chem. Soc. Chem. Commun.* **1991**, 1611 – 1612.
[26] M. del Mar Garcia-Molina, F. Muñoz-Muñoz, F. Garcia-Molina, P. A. García-Ruiz, F. Garcia-Canovas, *J. Agric. Food Chem.* **2012**, *60*, 6447 – 6453.
[27] L. M. Mirica, X. Ottenwaelder, T. D. P. Stack, *Chem. Rev.* **2004**, *104*, 1013 – 1045.
[28] E. A. Lewis, W. B. Tolman, *Chem. Rev.* **2004**, *104*, 1047 – 1076.
[29] R. S. Himmelwright, N. C. Eickman, C. D. LuBien, E. I. Solomon, *J. Am. Chem. Soc.* **1980**, *102*, 5378 – 5388.
[30] R. L. Martin, *J. Chem. Phys.* **2003**, *118*, 4775 – 4777.
[31] I. Sanyal, K. D. Karlin, R. W. Strange, N. J. Blackburn, *J. Am. Chem. Soc.* **1993**, *115*, 11259 – 11270.
[32] Materials and methods are described in the Supporting Information.
[33] S. Herres-Pawlis, P. Verma, R. Haase, P. Kang, C. T. Lyons, E. C. Wasinger, U. Flörke, G. Henkel, T. D. P. Stack, *J. Am. Chem. Soc.* **2009**, *131*, 1154 – 1169.
[34] S.-I. Yamazaki, S. Itoh, *J. Am. Chem. Soc.* **2003**, *125*, 13034 – 13035.
[35] J. L. Muñoz-Muñoz, J. Berna, M. del Mar Garcia-Molina, F. Garcia-Molina, P. Antonio García-Ruiz, R. Varon, J. N. Rodriguez-Lopez, F. Garcia-Canovas, *Biochem. Biophys. Res. Commun.* **2012**, *424*, 228 – 233.
[36] Z. Hu, R. D. Williams, D. Tran, T. G. Spiro, S. M. Gorun, *J. Am. Chem. Soc.* **2000**, *122*, 3556 – 3557.
[37] M. Kodera, K. Katayama, Y. Tachi, K. Kano, S. Hirota, S. Fujinami, M. Suzuki, *J. Am. Chem. Soc.* **1999**, *121*, 11006 – 11007.
[38] J. E. Bulkowski, US patent 4545937, **1984**.
[39] V. Kahn, N. Ben-Shalom, *Pigm. Cell Res.* **1998**, *11*, 24 – 33.
[40] W. W. Westerfeld, *Biochem. J.* **1940**, *34*, 51 – 58.

