tatin 2 (**2**) was identical in all respects (500 MHz $^{1}$H NMR, LR-MS, HR-MS, $[\alpha]_D^{25}$, TLC (3 solvent systems), and HPLC) with an authentic sample kindly provided by Professor Evans.[17]

In summary, the total synthesis of (+)-spongistatin 2 (**2**) has been achieved. Highlights of the synthesis include the stereocontrolled synthesis of the E and F bis(tetrahydropyran) subunit, Wittig coupling with an advanced ABCD fragment, and acid-mediated epimerization in the presence of a Ca$^{2+}$ ion of (−)-*epi*-spongistatin (**23**) to (+)-spongistatin 2 (**2**).

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[1] a) G. R. Pettit, Z. A. Cichacz, F. Gao, C. L. Herald, M. R. Boyd, J. M. Schmidt, J. N. A. Hooper, *J. Org. Chem.* **1993**, *58*, 1302; b) G. R. Pettit, *Pure Appl. Chem.* **1994**, *66*, 2271; c) R. Bai, G. F. Taylor, Z. A. Cichacz, C. L. Herald, J. A. Kepler, G. R. Pettit, E. Hamel, *Biochemistry* **1995**, *34*, 9714.

[2] A. B. Smith III, V. A. Doughty, Q. Lin, L. Zhuang, M. D. McBriar, A. M. Boldi, W. H. Moser, N. Murase, K. Nakayama, M. Sobukawa, *Angew. Chem.* **2001**, *113*, 197; *Angew. Chem. Int. Ed.* **2001**, *40*, 191.

[3] H. C. Brown, K. S. Bhat, *J. Am. Chem. Soc.* **1986**, *108*, 5919.

[4] T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974.

[5] J. J.-W. Duan, A. B. Smith III, *J. Org. Chem.* **1993**, *58*, 3703.

[6] P. A. Bartlett, J. D. Meadows, E. G. Brown, A. Morimoto, K. K. Jernstedt, *J. Org. Chem.* **1982**, *47*, 4013.

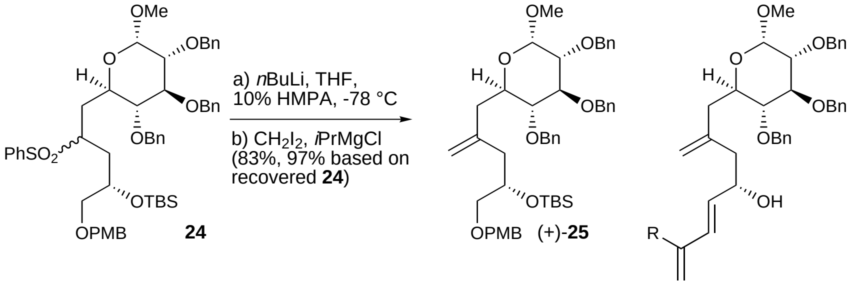
[7] A. B. Smith III, L. Zhuang, C. S. Brook, A. M. Boldi, M. D. McBriar, W. H. Moser, N. Murase, K. Nakayama, P. R. Verhoest, Q. Lin, *Tetrahedron Lett.* **1997**, *38*, 8667.

[8] Satisfactory spectroscopic data ($^{1}$H and $^{13}$C NMR, LR-MS, HR-MS, $[\alpha]_D^{25}$, and IR) were obtained for all new compounds.

[9] When a TBS group was used to protect the C(38) hydroxyl group, it could not be removed in the final step of the synthesis.

[10] A. B. Smith III, Q. Lin, G. R. Pettit, J.-C. Chapuis, J. M. Schmidt, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 567. In conjunction with the construction of spongistatin analogues (+)-**26a** and (+)-**26b**, Julia methylenation of **24** furnished (+)-**25** in excellent yield (83%).

[11] D. B. Dess, J. C. Martin, *J. Am. Chem. Soc.* **1991**, *113*, 7277.



(+)-**26a**, R = H, GI$_{50}$ 0.25 µg mL$^{-1}$, Pancreas-a BXPC-3
(+)-**26b**, R = Cl, GI$_{50}$ 3.2 µg mL$^{-1}$, Pancreas-a BXPC-3

[12] C. De Lima, M. Julia, J.-N. Verpeaux, *Synlett* **1992**, 133.

[13] M. M. Hayward, R. M. Roth, K. J. Duffy, P. I. Dalko, K. L. Stevens, J. Guo, Y. Kishi, *Angew. Chem.* **1998**, *110*, 202; *Angew. Chem. Int. Ed.* **1998**, *37*, 192.

[14] a) D. A. Evans, B. W. Trotter, B. Côté, P. J. Coleman, L. C. Dias, A. N. Tyler, *Angew. Chem.* **1997**, *109*, 2957; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2744; b) D. A. Evans, B. W. Trotter, P. J. Coleman, B. Côté, L. C. Dias, H. A. Rajapakse, A. N. Tyler, *Tetrahedron* **1999**, *55*, 8671; c) for an additional example of regioselective macrolactonization, see I. Paterson, K.-S. Yeung, R. A. Ward, J. D. Smith, J. G. Cumming, S. Lamboley, *Tetrahedron* **1995**, *51*, 9467.

[15] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.

[16] Kishi et al. reported that treatment of a protected 23-*epi*-macrocycle with HF · pyr. in pyr./THF furnished only 23-*epi*-spongistatin 1, which could not be epimerized to spongistatin 1 (**1**) upon treatment with either CSA/CH$_2$Cl$_2$ or HCl/CHCl$_3$, see J. Guo, K. J. Duffy, K. L. Stevens, P. I. Dalko, R. M. Roth, M. M. Hayward, Y. Kishi, *Angew. Chem.* **1998**, *110*, 198; *Angew. Chem. Int. Ed.* **1998**, *37*, 187.

[17] We thank Professor Evans for supplying an authentic sample of (+)-spongistatin 2 (**2**).

[18] Abbreviations used: Ac = acetyl; Bn = benzyl; TES = triethylsilyl; TBS = *tert*-butyldimethylsilyl; TMS = trimethylsilyl; BPS = *tert*-butylbiphenylsilyl; CSA = 10-camphorsulfonic acid; LiDBB = di-*tert*-butylbiphenyllithium; Piv, pivaloyl = 2,2-dimethylpropanoyl; DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone; TBAI = tetrabutylammonium iodide; Tf = triflate = trifluoromethanesulfonyl; imid. = imidazole; DMA = *N*,*N*-dimethylacetamide; DIBAL = diisobutylaluminum hydride; DMAP = 4-dimethylaminopyridine; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; Ts = tosyl; pyr = pyridine; HMPA = hexamethylphosphoramide; THF = tetrahydrofuran; HR-MS = high-resolution mass spectrometry; LR-MS = low-resolution mass spectrometry; TLC = thin-layer chromatography; HPLC = high-performance liquid chromatography.

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# A Chemical Model of Homeostasis**

Helmut H. Zepik, Eveline Blöchliger, and
Pier Luigi Luisi*

The experimental realization of self-replication systems[1–3] has permitted the representation of one of the key mechanisms of life in simple chemical terms. The chemical rendering of other main cellular mechanisms remains, however, elusive. One of these is homeostasis. There are different forms of homeostasis, and in quite general terms one may envisage that the chemical rendering of such a cellular feature might be constituted by a spherical bilayer structure which hosts chemical reactions and which is self-maintaining, because these reactions regenerate all components of the system that disappear in the reactions. One simple possible working unit of this kind, based on the structure of liposomes and on the notion of chemical autopoiesis,[4–7] was proposed a few years ago[8] and is shown in Figure 1 a.

This represents a spherical system with a semipermeable boundary formed by one single component S, into which the reagent

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A can permeate. Owing to the particular conditions inside the boundary, A is transformed by a single chemical reaction with velocity $v_p$ into the surfactant S, which is then sequestered into the membrane. A concurrent reaction destroys S, transforming it into P with velocity $v_d$. It is clear that this system permits various kinetic conditions (modes) which may simulate, although at the most primitive level, the overall behavior of a minimal cell. In fact, if the two velocities are numerically equal, the autopoietic unit is in homeostasis; that is, it maintains its identity and its concentration, despite and in force of the two concurrent reactions. If $v_d$ is larger than $v_p$, the autopoietic unit will be destroyed (simulating death). Finally, if the velocity of formation is larger than the velocity of decay, the system will tend to grow due to accumulation of the product S on the membrane. Self-reproduction may then occur if conditions of monodispersity of the spherical structures are imposed.

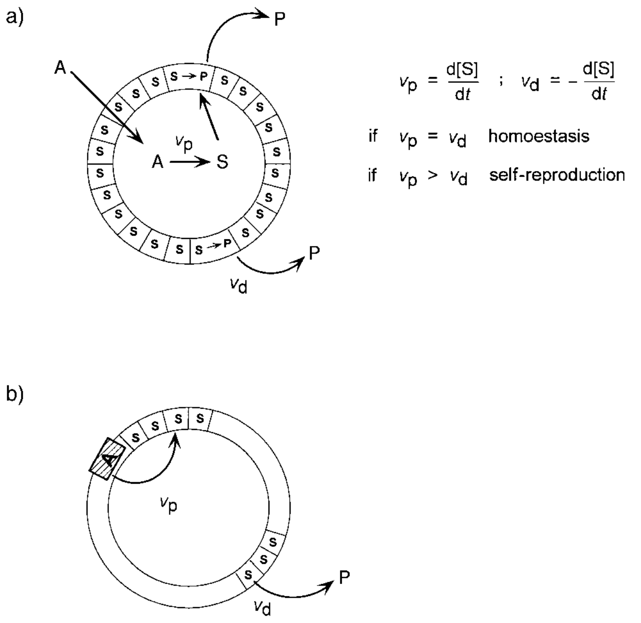

Figure 1. The simplest possible chemical rendering of the overall cell behavior (autopoietic unit), characterized by only two competitive reactions of anabolism and catabolism. The boundary is built by only one component S, which is continuously regenerated on the boundary with velocity $v_p$ from the nutrient A. A competitive reaction destroys S, transforming it into P with velocity $v_d$. Depending upon the relative values of the two velocities, the autopoietic unit can undergo various kinetic destinies, namely, homeostasis, growth, or death.[7] a) The ideal case in which the bilayer is permeable to the precursor A. b) The case in which the precursor A is water-insoluble and binds to the bilayer membrane, where it is transformed in situ into the surfactant S.

The question is now, how to transpose this model into experimental chemical terms. Spherically closed structures such as those represented in Figure 1 call to mind vesicles, and in fact liposomes (lipid vesicles) have been regarded as protocells, as prebiotic cellular shell structures.[9, 10] More recently, conditions have been described under which vesicles,[11, 12] as well as micelles,[3] are able to self-reproduce. It must be added that in all these systems the experimental implementation was based on a slight variation of the model in Figure 1a, which is shown in Figure 1b: In this case, the precursor of the surfactant S is a water-insoluble substance which does not penetrate into the aqueous core of the vesicle, but remains bound on the surface. Reactions are still confined within the boundary of the spherical aggregate and are determined by the properties of the aggregate itself, so that the conditions of autopoiesis[4–7] still hold.

Whereas self-reproduction based on the model in Figure 1 could be realized, the implementation of the homeostatic mode proved to be much more difficult and has not been described to date. We present here a simple system which is able to perform this task. This is a system which, depending on the initial parameters, is in principle capable of performing all the three modes represented in Figure 1, namely, homeostasis, self-reproduction, and “death”. To this aim, we have utilized the vesicular system oleic acid/oleate under the conditions of self-reproduction,[13, 14] but modified it by adding a reaction which destroys the vesicles by oxidizing the oleate double bond. At variance with previous systems, we will be dealing now with a continuously doubly fed system, in which both the oleic anhydride (feeding) and the oxidizing medium (destroying) are added separately by continuous injection. The whole system can be seen as a continuous reactor (Figure 2).

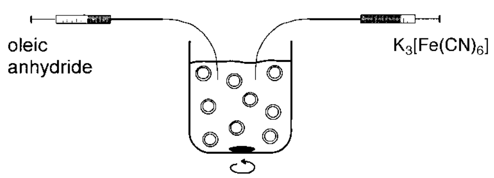

Figure 2. Schematic representation of the chemical bioreactor forming the experimental autopoietic model. The macroscopic system consists of the two injection syringes, each driven by a step motor (Bioblock Scientific KDS 100) to add oleic anhydride (2 μL h$^{-1}$) and 0.93 M $K_3[Fe(CN)_6]$ (50 μL h$^{-1}$) to a stirred suspension (20 mL, borate buffer (100 mM), pH 8.8) of oleate vesicles (10 mM oleic acid/oleate) charged with dihydroquinidine (0.5 mM) and $OsO_4$ (0.2 mM) at 40 °C.

Let us analyze in more detail the chemistry. As mentioned above, the feeding element is the water-insoluble oleic anhydride. This is dissolved into the preformed vesicles, and hydrolyzed on or in the bilayer, following the chemical scheme represented by Figure 1b. The surfactant produced in this way partly induces the growth of the host vesicles and partly induces the formation of new vesicles.[11, 12, 14] This increase of active surface facilitates further solubilization and hydrolysis of the anhydride: The more vesicles are produced, the more the anhydride is solubilized and hydrolyzed, in a typical autocatalytic process.

At the same time, the presence of oxidizing agents brings about a partial destruction of the vesicles. The oxidation reaction that we have used is the well-known $OsO_4$-catalyzed *cis* hydroxylation of olefins[15] with the hexacyanoiron(III) ion as a cooxidant.[16] Osmium tetroxide reacts first with the olefin to give a cyclic diester, which is then hydrolyzed to the diol and osmate ($OsO_4^{2-}$). The latter is continuously regenerated to $OsO_4$ by an excess of $K_3[Fe(CN)_6]$. The oxidation affords 9,10-dihydroxystearic acid, which does not form vesicles (data not shown).

The osmium tetroxide/ferrocyanide system is per se water-soluble. To ensure that also the oxidation reaction takes place on the bilayer, dihydroquinidine was added to the liposome suspension. This base is known to form strong complexes with $OsO_4$,[17, 18] and in fact it has been used in an aqueous–organic two-phase system, whereby the osmium tetroxide was kept in the organic phase.[19] We could show in a series of ultrafiltration experiments that when dihydroquinidine (0.5 mM) is added to a vesicle suspension (10 mM oleic acid/oleate) it is completely bound to the bilayers. As with the aqueous–organic two-phase system, the actual osmylation of the olefin occurs on the bilayer. The resulting osmium(VI) monoglycolate ester undergoes hydrolysis at the interface, releasing ligand to the bilayer, and diol and $OsO_4^{2-}$ to the aqueous pool.

The two competitive reactions are shown in Scheme 1. The reaction was carried out in a reaction vial that was fed continuously with anhydride and hexacyanoiron(III) (Figure 2). The two reactions were first studied individually in order to calibrate their individual rates (data not shown). Once conditions had been found under which the two rates were numerically equal, the oleate concentration was monitored as a function of time. In a typical experiment (Figure 3, curve a) a constant concentration of oleic acid/oleate could be kept for 20 h. This means that the concentration of vesicles remains constant, and in fact the turbidity of the vesicles suspension remains also constant (data not shown). Our earlier results[11, 12] indicate that under similar conditions, but without catalytic $OsO_4/K_3[Fe(CN)_6]$ and in the presence of preadded vesicles, the concentration of fatty acids and correspondingly the optical density increases by a factor of two within two hours. In principle, homeostasis conditions could be maintained for a much longer period of time.

a) $$[CH_3(CH_2)_7CH{=}CH(CH_2)_7CO]_2O + H_2O \longrightarrow 2\ CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$$

b) $$CH_3(CH_2)_7CH{=}CH{-}(CH_2)_7COOH + OsO_4 + 2\ OH^- \longrightarrow CH_3(CH_2)_7CH(OH)CH(OH)(CH_2)_7COOH + OsO_4^{2-}$$

Scheme 1. The two basic reactions of the autopoietic system: a) Hydrolysis of oleic acid anhydride in borate buffer (pH 8.8). b) Oxidation of oleic acid to 9,10-dihydroxystearic acid with cat. $OsO_4/K_3[Fe(CN)_6]$.

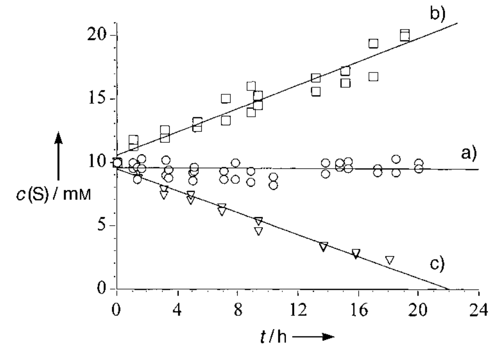

Figure 3. The time course of oleic acid generation in the system of Figure 2. a) Hydrolysis and oxidation (○); the velocity of hydrolysis and the velocity of oxidation (see Scheme 1) are numerically equal. b) Only hydrolysis (□); the velocity of oleate production (and therefore the formation of vesicles) is predominant. c) Only oxidation (▽); the decay velocity (the rate of oleate oxidation and breakdown) is predominant, which leads to the destruction of the whole system. The oleic acid concentration $c(S)$ was determined by GC analysis (Hewlett Packard 5890A, capillary Nukol column (Supleco)) after addition of 1M HCl and extraction into isooctane.

Curve b in Figure 3 shows the increase of oleic acid/oleate concentration and qualitatively illustrates the case in which the velocity of production of S is much larger than the velocity of oleic acid/oleate oxidation. Conversely, curve c shows the oxidation rate of oleic acid/oleate and illustrates qualitatively the case in which the velocity of vesicle destruction is much larger than the velocity of vesicle regeneration. Eventually the concentration of oleic acid/oleate decreases to zero (the solution becomes transparent).

In conclusion, we have realized the system of Figure 1, in the modification illustrated by Figure 1b. In fact, depending upon the conditions, either one of the three kinetic patterns of the minimal autopoietic system can prevail.

The model cannot be but very primitive, considering that it has been achieved on the basis of very simple chemical means, that is, hydrophobic forces and one hydrolysis and one oxidation reaction. Also, as a further limitation of this work, the model represented here corresponds to only one particular type of homeostasis. On the other hand, one can argue that the force of this model resides precisely in the simplicity of the used means: It is actually surprising that by such simple chemical means a homeostasis model can be realized.

The relevance of this simple model lies also in the fact that, based on these chemical principles, one can conceive more complex and more biologically relevant homeostatic systems (e.g., with internalized genetic material), which may permit one to get closer to a metabolic model of a cell. This does not appear out of reach, considering that biologically active ribosomes have been entrapped into liposomes,[20] and that quite complex molecular biological reactions do take place in vesicles.[21]

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[1] G. von Kiedrowski, B. Wlotzka, J. Helbing, *Angew. Chem.* **1989**, *101*, 1259–1261; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1235–1237.
[2] J. Rebek, Jr., *Experientia* **1991**, *47*, 1096–1104.
[3] P. A. Bachmann, P. L. Luisi, J. Lang, *Nature* **1992**, *357*, 57–59.
[4] F. J. Varela, H. R. Maturana, R. Uribe, *Biosystems* **1974**, *5*, 287–296.
[5] H. Maturana, F. J. Varela, *The Tree of Knowledge*, Shambhala, Boston, **1987**.
[6] G. R. Fleischaker, *Biosystems* **1988**, *22*, 37–49.
[7] P. L. Luisi in *Thinking About Biology* (Eds.: W. Stein, F. J. Varela), Addison-Wesley, Reading, MS, **1993**.
[8] P. L. Luisi, *Origins Life Evol. Biosphere* **1998**, *28*, 613–622.
[9] a) D. W. Deamer, *Origins Life Evol. Biosphere* **1986**, *17*, 3–25; b) G. Ourisson, Y. Nakatani, *Chem. Biol.* **1994**, *1*, 11–23.
[10] H. J. Morowitz, *Beginnings of Cellular Life: Metabolism Recapitulates Biogenesis*, Yale University Press, New Haven, CT, **1992**.
[11] P. Walde, R. Wick, M. Fresta, A. Mangone, P. L. Luisi, *J. Am. Chem. Soc.* **1994**, *116*, 11649–11654.
[12] K. Morigaki, S. Dallavalle, P. Walde, S. Colonna, P. L. Luisi, *J. Am. Chem. Soc.* **1997**, *119*, 292–301.

[13] The term self-replication is limited to linear structures whose replication is based on the template chemistry of nucleic acids; the term self-reproduction is a more general one, valid for all structures including micelles and vesicles; and the term autopoietic self-reproduction is for the particular case in which reactions leading to self-reproduction take place within the boundary of the reproductive unit (e.g. within the boundary of a vesicle).

[14] E. Blöchliger, M. Blocher, P. Walde, P. L. Luisi, *J. Phys. Chem. B* **1998**, *102*, 10383–10390.

[15] M. Schroeder, *Chem. Rev.* **1980**, *80*, 187–213.

[16] M. Minato, K. Yamamoto, J. Tsuji, *J. Org. Chem.* **1990**, *55*, 766–768.

[17] W. P. Griffith, A. C. Skapski, K. A. Woode, M. J. Wright, *Inorg. Chim. Acta* **1978**, *31*, L413–L414.

[18] H. C. Kolb, M. S. Van Nieuwenhze, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483–2547.

[19] H.-L. Kwong, C. Sorato, Y. Ogino, H. Chen, B. K. Sharpless, *Tetrahedron Lett.* **1990**, *31*, 2999–3002.

[20] T. Oberholzer, K. H. Nierhaus, P. L. Luisi, *Biochem. Biophys. Res. Commun.* **1999**, *261*, 238–241.

[21] T. Oberholzer, M. Albrizio, P. L. Luisi, *Chem. Biol.* **1995**, *2*, 677–682.

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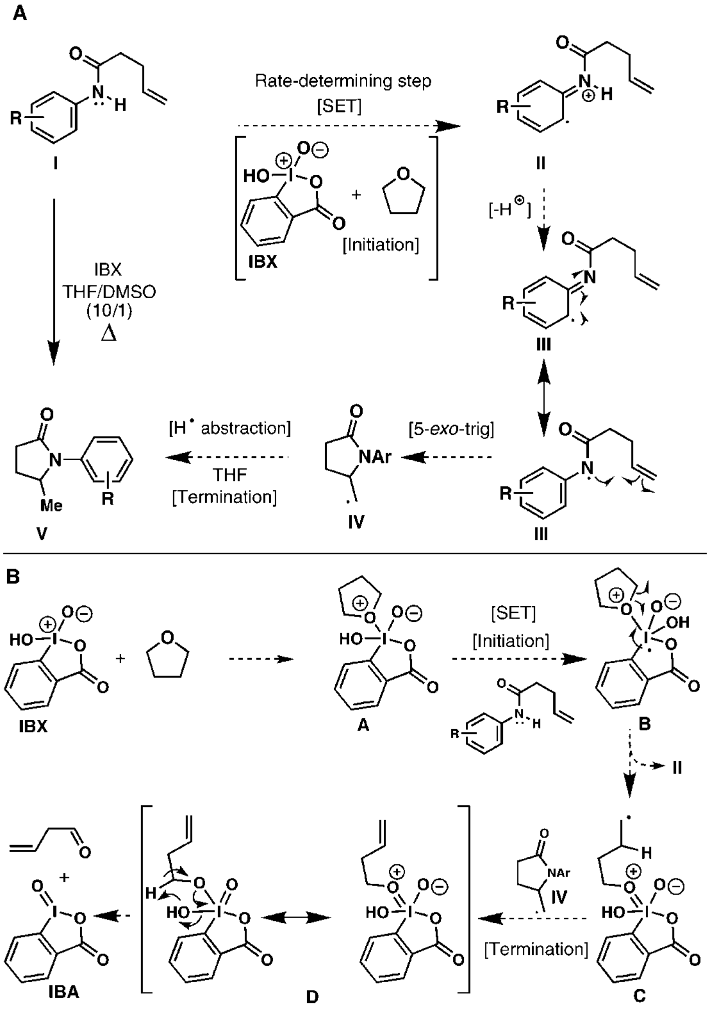
# Mechanistic Studies of Periodinane-Mediated Reactions of Anilides and Related Systems**

K. C. Nicolaou,* Phil S. Baran, Remo Kranich, Yong-Li Zhong, Kazuyuki Sugita, and Ning Zou

We have recently reported a series of novel synthetic technologies for the facile construction of complex polycycles,[1] diverse heterocycles,[2] amino-sugars,[3] and $\alpha,\beta$-unsaturated carbonyl compounds[4] induced by hypervalent iodine reagents (*o*-iodoxybenzoic acid (1-hydroxy-1,2-benziodoxol-3(1*H*)-one-1-oxide, IBX) and Dess–Martin periodinate (DMP)). This spate of reactions, which arose from a discovery made during the total synthesis of the CP molecules,[5] necessitated an in-depth mechanistic investigation to gain further understanding of the sequence of processes involved. Herein we present divergent mechanistic pathways for these IBX- and DMP-mediated reactions based on isotope labeling, kinetic studies, cyclic voltammetry measurements, NMR spectroscopic analysis, and designed cascade reactions.

For the IBX-mediated ring closures of anilides and related systems to N-heterocycles, we had previously proposed a pathway predicated on single electron transfer (SET) as shown in Scheme 1 (**I** →**II** →**III** →**IV** →**V**).[3] To confirm this hypothesis it was critical to first determine whether the hydrogen atom which must quench the proposed radical that would result from cyclization (**IV** →**V**, Scheme 1) originated from the substrate itself, IBX, or the solvent. Thus, when the reaction of **1a** (Scheme 2) with IBX was performed in $[D_8]$THF/$[D_6]$DMSO (10/1), deuterium was detected (NMR) in product [D]-**2a** at the carbon atom as predicted from the proposed mechanism. Carrying out the reaction of **1a** in THF/$[D_6]$DMSO (10/1) or of [D]-**1a** (see Scheme 2) in THF/DMSO (10/1) led to **2a** containing no deuterium. When the experiment was carried out without a hydrogen-donating solvent (such as THF or dioxane) present (for example, in neat DMSO), no reaction was observed.[6] This peculiar solvent dependence led us to conclude that the solvent actually plays a role in this reaction, besides being a source of



Scheme 1. Proposed mechanism of the IBX-mediated ring closure of anilides and related systems to N-heterocycles (**I**→**V**). SET = single electron transfer, IBX = *o*-iodoxybenzoic acid.

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