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Paclitaxel from Primary Taxanes: A Perspective on Creative Invention in Organozirconium Chemistry

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In this Perspective, which describes the achievements recognized by the 2007 ACS Award for Creative Invention, we discuss the discovery of a new synthetic reaction and its translation into a substantially improved method for manufacturing a major pharmaceutical product—the blockbuster anticancer drug, paclitaxel. The role of creativity in the discovery and invention processes is also discussed. As is often the case, chance discovery and serendipitous findings played a role in the evolution of this work. Translation of the basic research into a commercially viable paclitaxel semisynthesis is also described. The final manufacturing process illustrates the enormous impact that the globalization of markets has had on chemical and pharmaceutical manufacturing.

Where observation is concerned, chance favors only the prepared mind.

—Louis Pasteur

Necessity is the mother of invention.

—anonymous Latin saying

Git 'er done!

—Southern country/male expression (recently popularized by Larry, the Cable Guy)

The role of creativity in driving discovery and invention has long fascinated most scientists and engineers. Often (perhaps too simply) described as either pure or practical (i.e., translational) research, the desire to discover and to make things kindles, to varying degrees, inside all of us. And although we may each acknowledge and respond to those drives differently, that is not because we lack a passion for either activity. Describing the intrinsic attraction of basic research in organic synthesis, E.J. Corey once wrote, "the appeal of a problem in synthesis and its attractiveness can be expected to reach a level out of all proportion to practical considerations whenever it presents a clear challenge to the creativity, originality, and imagination of the expert in synthesis."[1] Echoing that view from the translational side of research, Jerome Lemelson, the prolific innovator and inventor who averaged one patent per month for more than 40 years, was fond of saying "I am always looking for problems to solve."[2]

The path from discovery to invention is often a convoluted and tortuous one, as open-ended ideas coalesce and take shape, ultimately to be implemented in new designs and/or new technologies. Serendipity and chance can play important roles, and so can the unrelenting drive to address an unmet need ("git 'er done") or, as Lemelson remarked, to crack an unsolved problem. We have been fortunate to have participated, at various stages, in the discovery of a new synthetic reaction and its implementation in a substantially improved method for manufacturing a major pharmaceutical product—the anticancer drug paclitaxel. This Perspective provides an opportunity to describe the path from basic discovery to commercial production, as well as to discuss the scientific and technical underpinnings of our achievement.

Taxol, known generically as paclitaxel, is one of the most successful anticancer agents to have been developed over the past 50 years. First approved by the FDA for ovarian cancer in 1992, paclitaxel was subsequently endorsed for breast cancer in 1994. In 1999, worldwide sales of the branded pharmaceutical by Bristol-Myers Squibb (BMS), which developed Taxol, achieved $1.5 billion in sales. Despite a several fold reduction in market price driven by competition from (now-generic) paclitaxel for injection and new indications for broader use, the

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(1) Corey, E. J. *Chem. Soc Rev.* **1988**, *17*, 111−133.

(2) Biography, Jerome Lemelson, published by the Lemelson Foundation; http://web.mit.edu/invent/w-lemelsonbio.html.

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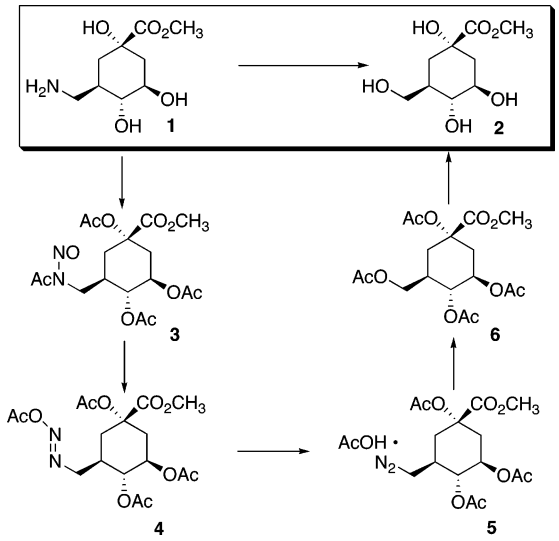
SCHEME 1



total market for paclitaxel remains well above $1 billion per year. Worldwide use of generic paclitaxel continues to expand, and additional exciting new clinical uses are anticipated.

Initial supplies of paclitaxel were severely restricted, and shortages of the drug hampered its preclinical and clinical development. One of nature's scarcest natural products, paclitaxel was first discovered in the Pacific yew, whose extinction was threatened by the harvesting of its bark. Major environmental concerns eventually led to more promising semisynthetic approaches to the drug.

The Potier group in France discovered that 10-deacetylbaccatin III (10-DAB) was more abundant than paclitaxel and could be harvested from the needles of the English yew. However, the transformation of 10-DAB into paclitaxel involved a difficult, multistep procedure. Independently, the Holton group at Florida State University developed an alternative solution to the side-chain attachment problem. That method was licensed to BMS and eventually commercialized.

Although paclitaxel has been available in generic form since 2000, the price of the drug remains high, in part because of the substantial complexity and cost of semisynthetic production using 10-DAB isolated from the English yew. Numerous accounts have already been published chronicling the history of paclitaxel drug development, to which readers are referred for more detailed information.[3,4] In this Perspective, we focus instead on the discovery and development of novel synthetic methodology employing zirconium-based reagents and its implementation in a short and efficient semisynthesis of paclitaxel from an extract of primary taxanes derived from nursery-grown ornamental yew trees.

The fundamental research that made this achievement possible could not have had a more obscure beginning! As part of a project aimed at the synthesis of a shikimate pathway inhibitor, the Cornell group needed to transform primary amine **1** into the corresponding alcohol **2** (Scheme 1).[5] While the converse transformation of alcohols into amines can be achieved using various nucleophilic substitution protocols, replacing an aliphatic

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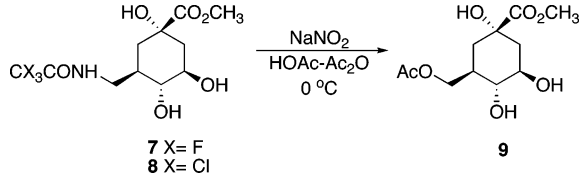

NH_2 group with OH poses a more demanding challenge, especially under conditions that would be compatible with other functionality present in **1**. A method of particular interest was the thermal rearrangement of *N*-nitrosocarboxamides to carboxylic esters, a process first studied by Huisgen et al.[6] and developed by White et al.[7]

To implement that process, published conditions called for heating the preformed peracetylated *N*-nitrosoamide **3** for several hours in a nonpolar solvent like hexane, whereupon **3** formed tetraester **6** via transient diazotate ester **4** and diazoalkane **5**. Exhaustive saponification of tetraacetate **6** afforded the desired alcohol **2**. A variation of that procedure utilizing milder, more selective conditions was developed at Cornell in which nitrosation of the corresponding unprotected trifluoro or trichloroacetamides **7** or **8**, respectively, at 0 °C in acetic acid triggered spontaneous rearrangement to monoacetate **9** without isolation of any intermediates (Scheme 2).[8] Besides being simple and efficient, the technique was compatible with many functional groups.

The remarkable series of intermediates leading from nitrosocarboxamides **7** or **8** to carboxylate ester **9** could also be exploited further. Of particular interest was the potential for developing new chemistry of alkanediazotate esters (e.g., **4**) and their congeners. For example, we demonstrated that by analogy to *N*-nitrosocarboxamides, the corresponding *N*-nitrosophosphoramides also rearranged smoothly to phosphoesters, presumably by way of the corresponding phosphorylated diazotate, thus achieving the first direct transformation of amines to primary alkyl phosphate esters.[9] Moreover, under highly nucleophilic, more dissociating conditions (trimethylamine *N*-oxide/DMSO), the rearrangement of *N*-nitrosocarboxamides to esters could be redirected to form aldehydes.[10]

Our interest in exploiting diazotates and their derivatives was further heightened by the ready availability of alkali metal diazotate salts. For example, both *cis* and *trans*-isomers of potassium diazotate salts **12** and **15** could readily be prepared by published methods (Scheme 3).[11] Using this chemistry, we further established that O-acylation of **12** and **15** provided an alternative route to the same diazotate esters implicated by Huisgen and White in the classical thermal rearrangements of nitrosoamides.[10]

(3) (a) Kingston, D. G. I. *J. Nat. Prod.* **2000**, *63*, 726−34. (b) Ojima, I.; Lin, S.; Wang, T. *Curr. Med. Chem.* **1999**, *6*, 927−954. (c) Cragg, G. M. *Med. Res. Rev.* **1998**, *18*, 315−331.

(4) (a) Kingston, D. G. I. *Trends Biotechnol.* **1994**, *12*, 222−227. (b) Horwitz, S. B. *J. Nat. Prod.* **2004**, *67*, 136−138.

(5) Nikolaides, N.; Ganem, B. *Tetrahedron Lett.* **1989**, *30*, 1461.

(6) (a) Huisgen, R.; Horeld, G. *Justus Liebigs Ann. Chem.* **1948**, *567*, 137. (b) Huisgen, R.; Krause, L. *Justus Liebigs Ann. Chem.* **1951**, *574*, 157. (c) Huisgen, R.; Reimlinger, H. *Justus Liebigs Ann. Chem.* **1956**, *599*, 183.

(7) (a) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6011, 6014. (b) White, E. H.; Aufdermarsh, C. A. *J. Am. Chem. Soc.* **1961**, *83*, 1174, 1179. (c) White, E. H.; DePinto, J. T.; Polito, A. J.; Bauer, I.; Roswell, D. F. *J. Am. Chem. Soc.* **1988**, *110*, 3708.

(8) Nikolaides, N.; Ganem, B. *J. Org. Chem.* **1989**, *54*, 5996.

(9) (a) Nikolaides, N.; Ganem, B. *Tetrahedron Lett.* **1990**, *31*, 1113. (b) Nikolaides, N.; Schipor, I.; Ganem, B. *Org. Synth.* **1993**, *72*, 246.

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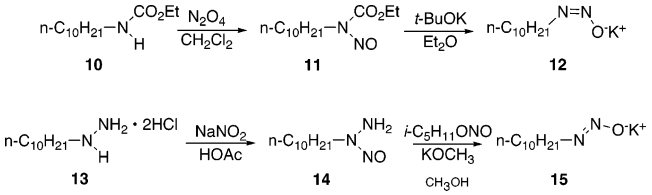
(11) Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421.

SCHEME 3



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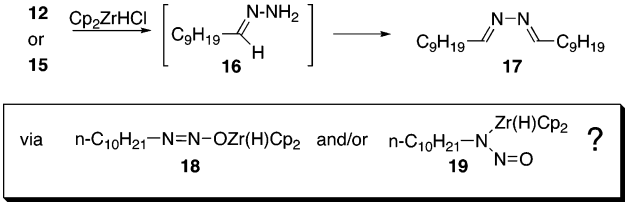


Our research took a new and unforeseen turn when we investigated the reaction of potassium diazotates **12** and **15** with organotransition metal halides. Although our general direction was by design, the valuable discovery that lay ahead was not on any chart of our course.

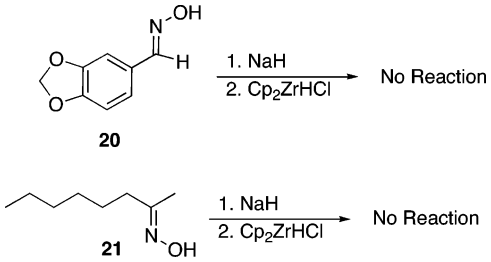
We decided to focus on the chemistry of early transition metals (e.g., titanium, zirconium, hafnium) whose strong metal−oxygen bonds might drive interesting deoxygenations, rearrangements, or other novel chemistry. The reductive coupling of dicarbonyl compounds to alkenes (the McMurry reaction),[12] which employs low-valent titanium, is a well-known and useful example of such a process.

Many low-valent zirconium complexes, typically Zr(IV), were known to display synthetically interesting chemistry that could be grouped into two broad categories. One was hydrozirconation[13] which, like hydroboration, involves stereoselective *cis*-addition of Cp_2ZrHCl to alkenes and alkynes, whereupon the resulting alkyl- or alkenyl-Cp_2ZrCl complexes can undergo useful coupling or alkyl transfer reactions. The other was the coupling of alkenes and alkynes to a wide variety of unsaturated ligands via zirconocene (Cp_2Zr) complexes to furnish highly functionalized carbocyclic and heterocyclic systems.[14] A somewhat related family of C−C bond-forming reactions developed by Erker involved Fischer-type carbene complexes derived from zirconocene.[15]

We began our investigation of deoxygenation pathways using Cp_2ZrHCl, also known as Schwartz's reagent. Reaction of *cis*-diazotate salt **12** with Cp_2ZrHCl in hexamethylphosphoric triamide (HMPA) at rt for 6 h afforded *n*-decylazine **17** in 66% yield (Scheme 4). Quenching the reaction after 1 h led to hydrazone **16**, which upon standing was gradually transformed into **17**, suggesting that **16** was the primary reaction product. The *trans*-diazotate salt **15** less efficiently afforded **17** (32%) together with 1-decanol (43%). The mechanism of this interesting deoxygenation is unknown, although initial formation of O- and/or N-zirconated intermediates such as **18** and **19** seems likely.

Attempts to deoxygenate aldoximes or ketoximes by a similar procedure were unsuccessful. Reaction of **20** or **21** (Scheme 5)

SCHEME 5



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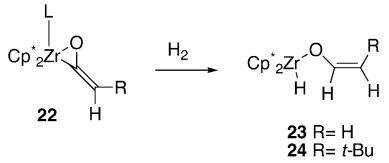
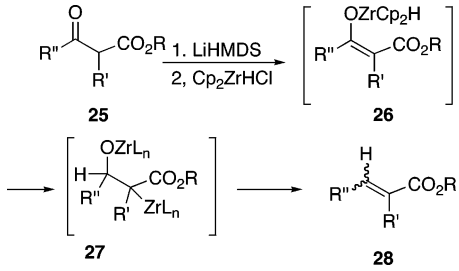


SCHEME 7



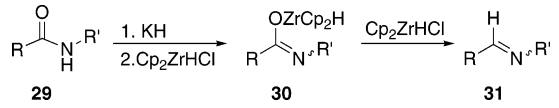
with sodium hydride and then with Schwartz's reagent returned both oximes, even after heating at 100 °C.

The deoxygenation of carbonyl compounds by way of their hydridozirconocene enolates was another area of active investigation within the Cornell group. Little was known at the time about the chemistry of such species. While chlorozirconocene enolates were reported in 1980,[16] the first "zirconium enolate hydrides" *cis*-**23** and *cis*-**24** (Scheme 6) were prepared in 1983 by an indirect approach from **22** and were stable enough to be characterized crystallographically.[17]

Since enolates **23** and **24** showed no tendency to deoxygenate, we envisioned the alternative possibility that hydridozirconium species derived from β-dicarbonyl compounds and other extended enolate systems might undergo deoxygenation by an addition−elimination pathway. In fact, transformation of β-ketoesters like **25** into their hydridozirconocene enolates **26** using base and Cp_2ZrHCl efficiently formed α,β-unsaturated esters **28** in a one-pot reaction (Scheme 7).[18] The reaction mechanism of this heterogeneous process likely involved regioselective intermolecular hydrozirconation of the conjugated alkene in **26**, followed by a well-precedented[19] β-elimination of the Zr−O leaving group in **27** to furnish **28**. β-Diketones like 1,3-cyclohexanedione underwent a similar transformation, albeit in lower yield because the product cyclohexenone was susceptible to further addition reactions. The intermediacy of enolate **26** was established by independent synthesis (from the correspond-

(12) McMurry, J. E. *Acc. Chem. Res.* **1983**, *16*, 405.
(13) Schwartz, J.; Labinger, J. A. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 333.
(14) Broene, R. D.; Buchwald, S. L. *Science* **1993**, *261*, 1696.
(15) Erker, G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 397.
(16) (a) Evans, D. A.; McGee, L. R. *Tetrahedron Lett.* **1980**, *21*, 3975. (b) Evans, D. A.; McGee, L. R. *J. Am. Chem. Soc.* **1981**, *103*, 2876.
(17) Moore, E. J.; Straus, D. A.; Armantrout, J.; Santarsiero, B. D.; Grubbs, R. H.; Bercaw, J. E. *J. Am. Chem. Soc.* **1983**, *105*, 2068.
(18) Godfrey, A. G.; Ganem, B. *Tetrahedron Lett.* **1992**, *33*, 7461.
(19) Wailes, P. C.; Weigold, H.; Bell, A. P. *J. Organometal. Chem.* **1972**, *43*, C32.

SCHEME 8



ing chlorozirconium enolate by $LiBEt_3H$ reduction); **26** directly afforded **28**. Overall, the one-pot process circumvented the traditional 3-step sequence (reduction, mesylation/tosylation, and elimination) used to transform β-ketoesters to α,β-enoates.[5]

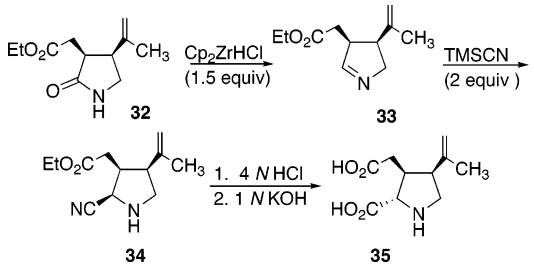
The successful deoxygenation of conjugated enolates not only vindicated our investment in this line of research but also fueled our interest in exploring other electron-deficient alkenes or related π-systems capable of similar reactions with Schwartz's reagent. A fateful choice was made to investigate hydridozirconium species like **30** (accessible by metalation of secondary amides like **29**, Scheme 8), which might undergo hydrozirconation/elimination to afford N-substituted imines **31**. It was unclear at the outset whether a hydrolytically sensitive, easily reduced imine like **31** could be isolated or whether it would be further reduced to the amine stage. However, we forged ahead, since an efficient nonaqueous workup had been developed in earlier studies on enoates that would suppress imine hydrolysis and since reduction of an uncoordinated or weakly coordinated imine might be sluggish.

In the event, the method for reducing secondary amides to imines shown in Scheme 8 was smoothly reduced to practice. For ordinary amides, reduction was best achieved by an initial KH metalation, followed by treatment with Cp_2ZrHCl. For relatively acidic carboxamides, metalation, and reduction was accomplished directly using Cp_2ZrHCl.[20] In both procedures, optimal yields of imine required two equiv of Schwartz's reagent, suggesting that the intermediate hydridozirconocene **30** underwent reduction by Cp_2ZrHCl by way of dinuclear complexes similar to those observed in carbonylation reactions of zirconium hydrides.[21]

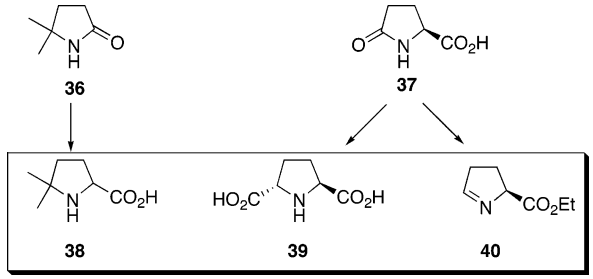
Even before this unique amide-to-imine transformation was implemented in any commercial process, the Cornell group was mindful of its potential industrial utility, and anticipated potential limitations of the method. To quote from the 1996 full paper on this methodology, "From a practical standpoint, however, the overall requirement for 2 mol equiv of Cp_2ZrHCl was a major disadvantage of the method, posing cost and disposal problems on a large scale." The laboratory began investigating variations designed to reduce or eliminate the need for Schwartz's reagent. In fact, it proved possible to replace hydridozirconocene enolate **30** with the corresponding diisobutylaluminum enolate, which was also smoothly reduced to imine **31** using only 1 equiv of Cp_2ZrHCl. In retrospect, however, the concerns expressed never materialized. Numerous zirconium compounds, including aluminum zirconium chloride and its congeners, were already widely used commercially as ingredients in antiperspirants and other personal care products.

Since its publication, the partial reduction of amides and lactams using Cp_2ZrHCl has found application in a number of interesting transformations that take advantage of the product

SCHEME 9



SCHEME 10



imines by various means. In 2001, an asymmetric total synthesis of the excitatory aminoacid, kainic acid **35** (Scheme 9), a scarce commodity at the time, was achieved using the method.[22] The synthetic plan involved zirconium-mediated reduction of 3,4-disubstituted butyrolactam **32** to the corresponding imine **33**. Without isolation, that imine was then subjected to a Strecker reaction to create the necessary α-aminoacid functionality in **34**. Hydrolysis and epimerization of **34** afforded (−)-α-kainic acid.

The amide-to-imine reduction has also been used to achieve efficient syntheses of other substituted pyrrolidines, such as the conformationally constrained amino acid 5,5-dimethylproline **38** (Scheme 10), the marine metabolite (*2S,5S*)-pyrrolidine-2,5-carboxylic acid **39**, and glutamic semialdehyde ethyl ester **40**, from readily available 2-pyrrolidinones **36** and **37**.[23]

Contemporaneous with development of the new amide-to-imine reduction at Cornell, the commercial production of paclitaxel from its natural yew tree sources was shifting into high gear. Following human clinical trials that established paclitaxel's efficacy against ovarian and breast cancer, Bristol-Myers Squibb faced numerous challenges in bringing the newly approved drug to the market. the original source of paclitaxel, the bark of the Pacific yew (which was extracted under contract with Hauser, Inc.), was not only environmentally unsustainable, but was inadequate to meet the expected market demand. After exploring alternative sources of the drug, BMS eventually terminated its agreement with Hauser in favor of a semisynthetic process developed at Florida State that transformed 10-DAB (obtained from the leaves of the European yew tree) into paclitaxel.

In 1999, Natural Pharmaceuticals, Inc. (NPI) acquired most of the assets of Hauser's oncology division. Included in Hauser's patent estate was US Patent 5,679,807, which applied the Cornell amide-to-imine reduction to remove the amide side chain from a mixture of primary taxanes extracted from the yew.[24] NPI

(20) (a) Schedler, D. A.; Godfrey, A. G.; Ganem, B. *Tetrahedron Lett.* **1993**, *34*, 5035. (b) Schedler, D. A.; Li, J.; Ganem, B *J. Org. Chem.* **1996**, *61*, 4115.

(21) (a) Wolczanski, P. T.; Bercaw, J. E. *Acc. Chem. Res.* **1980**, *13*, 121. (b) Erker, G. *Acc. Chem. Res.* **1980**, *17*, 103.

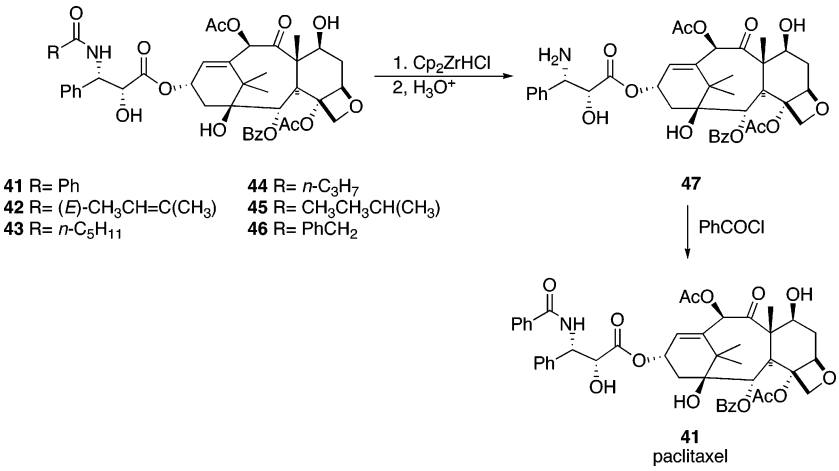
(22) Xia, Q.; Ganem, B. *Org. Lett.* **2001**, *3*, 485.

(23) Xia, Q.; Ganem, B. *Tetrahedron Lett.* **2002**, *43*, 1597.

(24) Murray, C. K.; Zheng, Q. Y.; Cheng, X.; Peterson, S. K. U.S. Patent 5,679,807; *Chem. Abstr.* **1996**, *125*, 222220.

FIGURE 1. Harvesting the initial biomass for extraction of primary taxanes. Pruned tree tops of 8−10-year-old nursery-grown ornamental yew trees on Michigan's Upper Peninsula will regenerate after 3−4 years.

SCHEME 11



subsequently acquired contracts to some 3.5 million nursery-grown ornamental yew trees growing on Michigan's Upper Peninsula. Unlike the very slow-growing Pacific yew, which required 100−200 years to reach full size, these robust, shrub-sized ornamental yews were environmentally unthreatened. They matured in just a few years and could easily be regenerated from branch cuttings. Of particular significance was the fact that ornamental yew trees contained substantial quantities of predominantly six primary taxane constituents **41**−**46** (Scheme 11) that shared the identical tetracyclic ring nucleus of paclitaxel itself and differed only in the amide bond residue of the side chain. Reduction of those amides using Schwartz's reagent and hydrolysis of the corresponding mixture of imines should afford a common primary amine **47**. Simple benzoylation of **47** would then furnish paclitaxel **41**.

Armed with that patent, NPI implemented and optimized the streamlined and highly convergent semisynthetic process outlined in Scheme 11. By appropriate modification of solvent, temperature, reagent stoichiometry, and workup conditions, NPI scientists demonstrated for the first time that it was possible to produce bulk quantities of high-purity paclitaxel from primary taxanes consistently and in a cost-effective manner. The new route to paclitaxel is currently competitive with existing production pathways.

From nursery-grown yews to final finished product, the NPI route to paclitaxel is a testament to the impact of emerging international trade and globalization on chemical and pharmaceutical manufacturing. The process begins in Michigan, where yew trees, typically 8−10 years old, are harvested in a sustainable fashion by pruning ca. 30 cm from the tree tops (Figure 1). Pruned trees regenerate their tops after 2 years, and branch cuttings can be used to grow 1−2 million new yew trees per year.

The resulting tops are dried within 5 h of harvesting. When fields grow to confluency the whole tree, including the root stock, is harvested. The dryer, which is heated using sawdust obtained free from a nearby furniture factory, can produce more than 100 tons of dry biomass per day (Figure 2).

The dried powder from treetops and other yew biomass is then pelletized (Figure 3), which not only reduces transportation costs but also increases the efficiency of the subsequent extraction process.

The pellets are then shipped (Figure 3) to a retrofitted marigold extraction plant in Mexico for isolation of the crude

FIGURE 2. Large-scale drying of the biomass occurs within 5 h of harvesting and can produce up to 100 tons of dried plant material per day.

FIGURE 3. Powdered biomass (shown at top) produced in Michigan is pelletized (middle) and then shipped to Mexico for extraction. The powdered extract (shown at bottom) of primary taxanes contains 10% paclitaxel by weight.

taxanes. Up to 8 tons of pelletized biomass can be extracted per day, with solvent recycling to reduce costs and environmental impact. The extraction affords a brown powder (Figure 3) containing 10% paclitaxel by weight.

Continuing the multinational refinement process, the crude paclitaxel extract is then shipped to Vancouver, Canada, where a wholly owned subsidiary of NPI conducts further isolation and purification of the primary taxane constituents. The three-stage process ultimately produces a blend of **41**−**46** in >95% purity. The conversion of primary taxanes to paclitaxel by selective N-benzoylation is performed by a wholly owned NPI subsidiary located in Shanghai, China, and with a contract manufacturer in Wisconsin.

In summary, a basic research discovery at Cornell led to a unique method for reducing amides selectively to imines. The method played a central role in the development and commercialization by NPI of a short and efficient new semi-synthesis of paclitaxel from the primary taxanes that are abundant in ornamental yew trees. As is evident from this Perspective, the path to the key discovery was a convoluted one (although perhaps not tortuous!) and involved a combination of focused exploration and empirical experimentation. Serendipity no doubt played a significant part in our findings; we felt certain we could expect something interesting would be discovered in our experiments—we just did not know exactly what to expect!

By contrast, the drive to develop a more efficient paclitaxel production route was triggered by a clear, logical analysis of the looming need for larger quantities of the drug. That motivation was also inspired by the relentless and unforgiving forces of the marketplace, where success would be achieved and measured only by the ability to produce the drug in high purity at the right cost so it could be sold at a competitive price. In every aspect of the manufacturing process, from securing bulk nursery contracts to recycling sawdust for fuel to a singular focus on achieving the highest possible yields and purity in the synthetic process, the NPI team demonstrated the fortitude ("git 'er done") necessary to meet those constraints.

Prospects are promising for continued growth in the generic paclitaxel market, both in the US as well as in Europe, Asia, and the Far East. Several additional developments are fueling

the increased demand for the drug, including novel formulations such as paclitaxel nanoparticles coated by albumin, a vitamin E based formulation, and other novel lipid formulations as well as taxanes derivatized with fatty acids or peptides to reduce side effects or enhance tumor targeting. Among the innovative applications are paclitaxel-coated stents to lower the incidence rate of restenosis afer coronary artery angioplasty.

Acknowledgment. It is with great pleasure that we acknowledge the extraordinary contributions of our colleagues and coworkers at Cornell and NPI whose creativity and invention made this undertaking a success. The Cornell team included Dr. Alexander Godfrey, Dr. David Schedler, Dr. Jun Li, Dr. Nick Nikolaides, Dr. Qian Xia, and Ms. Ioanna Schipor. We wish to acknowledge the vital contributions of Dr. James Johnson, who as the first employee at NPI took the early lead on this project. The NPI team also included Dr. Rex Gallagher, Dr. John Juchum, Dr. Jen Jiezhi Wang, Dr. Dasheng Wang, Dr. Susan Gong, Dr. C.T. Ho, and Mrs. Paula Fronce. We also thank our mutual friend and colleague, Professor Thomas P. Sakmar of Rockefeller University, for introducing us—a serendipitous event that forged the academic−industrial partnership in which this creative invention blossomed. Cover art by Karina Åberg.

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