Ozonolysis Applications in Drug Synthesis

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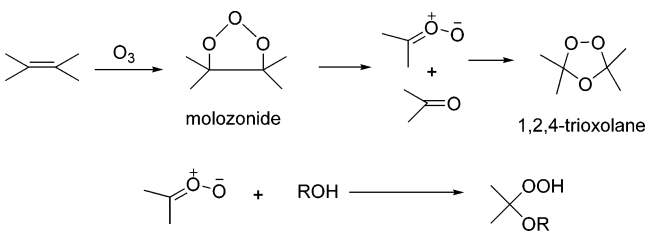
1. Introduction

Ozonolysis chemistry has been used extensively in academic, research, and industrial environments. A review on ozonolysis by Bailey appeared in *Chemical Reviews* nearly 50 years ago, and a significant amount of research has since been published and reviewed.[1−3] Synthetic chemists have many alternative chemical transformations at their disposal, but the ease with which an ozonolysis reaction can be conducted renders it a clean and effective choice for oxidative cleavage of double bonds. The primary concern with ozonolysis chemistry rests on safety issues because the low-molecular-weight ozonides and peroxides produced are unstable intermediates. These products could form an explosive hazard upon concentration during the workup if methods to detect and safely quench the peroxides are not employed. Scaling an ozonolysis reaction to an industrial level requires a careful assessment of the energetics of the reaction at hand. The mechanism of an ozonolysis reaction has been thoroughly studied, and the Criegee mechanism has been accepted as shown below.[4−6]



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Generally, ozone is generated from air or oxygen and passed through a cold solution (from 0 to −78 °C) of solvent and substrate until a blue color is observed, indicating destruction of the double bond. Typical solvents include methanol, ethyl acetate, dichloromethane, ethanol, water, and acetic acid. After sparging with nitrogen to dissipate the free ozone, a reductive or oxidative workup can afford a wide variety of products including alcohols, aldehydes, ketones, acids, and amines. Kula has reported conditions that allow for safely performing ozonolysis reactions up to a 500 g scale.[7] Because the high-energy ozonide (also called normal or secondary ozonide or 1,2,4-trioxolane) is formed in nonparticipating solvents, the use of protic solvents such as alcohols or acids is preferred. The resulting alkoxy and acyloxy hydroperoxides are reduced more efficiently and pose less of a safety risk during scale-up. The temperature at which an ozonolysis reaction is typically carried out is −78 °C; however, Kula recommends temperatures between 0 and −20 °C or higher. His reasoning is based on the idea that at higher temperatures the primary ozonide (1,2,3-trioxolane or molozonide) will be more likely to smoothly decompose into the corresponding hydroperoxides and disfavor collapse into the 1,2,4-trioxolane. Running the reaction at higher temperatures in participating solvents will promote the formation of hydroperoxide intermediates that can quickly react with the solvent and provide a safer reaction.

The reduction phase of an ozonolysis reaction is not only important from a safety standpoint but can also determine the product composition. Reductive workups can afford aldehydes, ketones, or alcohols. A substantial number of reducing reagents have been employed including zinc−acetic acid, sulfite ion, bisulfite ion, iodide, dimethyl sulfide, thiourea, and lithium aluminum hydride or sodium borohydride to afford alcohols.[1−2,8] The reductions themselves are generally exothermic and dose-controlled, requiring efficient cooling to dissipate the heat of reaction. The use of dimethyl sulfide offers several advantages including the ability to safely reduce peroxides to carbonyl products, and the excess sulfide can be removed by evaporation, provided that an efficient scrubbing system is in place.[9,10] Dimethyl sulfide is also a milder reducing reagent causing less of an exothermic event.[11] Trimethyl phosphite also has been used as an efficient reducing agent for quenching ozonolysis reactions and offers the advantage of less odor than dimethyl sulfide. Oxidative workups generally convert peroxide intermediates to ketones or carboxylic acids. Typical reagents include peroxy acids, silver oxide, chromic acid, oxygen, permanganate, or hydrogen peroxide.[1−2,8]

Additional transformations that broaden the scope of ozonolysis can be seen in the following reactions (Scheme

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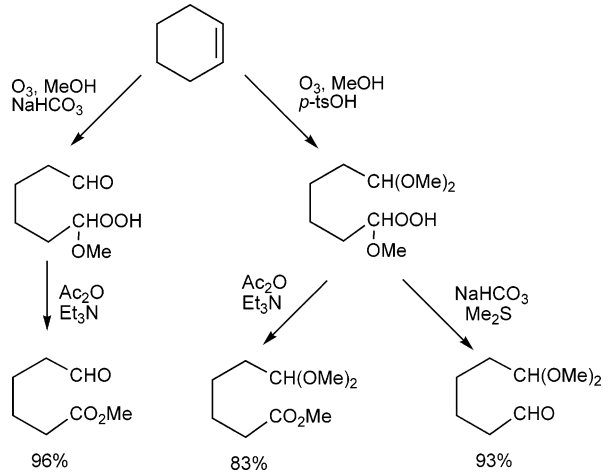
1). Schreiber has shown that by modifying the ozonolysis and workup procedures a variety of terminally functionalized products can be obtained from cycloalkenes.[12] When an acid or base is simply incorporated during the ozonolysis sequence followed by a reductive workup, mixed aldehydes, esters, and acetals can be furnished in high yields. The authors of this review carried out similar transformations using cyclic olefins on a 500 g scale to provide the acetal−aldehyde product in high yields (>70%).[13]

Reactions shown in Scheme 2 further demonstrate the use of ozone to provide a selective oxidative cleavage of silyloxyalkene **1** to lactone **2** in 93% yield.[14,15] The nucleophilicity of the silyloxyalkene double bond makes it more prone to ozonolysis than the less activated vinyl double bond. Ketene dithioacetal **3** can be efficiently cleaved with ozone to provide the corresponding ketone **4**.[16] The reaction of α,β-unsaturated ketone **5** with ozone affords keto acid **6** in 55% and is an important reaction in steroid chemistry.[17]

Richard J. Pariza is Vice President of Research and Development at Cedarburg Pharmaceuticals, Inc. He received his B.S. degree in chemistry from the University of Wisconsin—Milwaukee and a Ph.D. from Purdue University, working for Professor Philip Fuchs. He built and managed two process research departments at Abbott Laboratories and has consulted and worked for several small pharmaceutical companies. His chemical interests range from liquid-state interactions to the synthesis of natural and sophisticated (unnatural) products. He has chaired a Gordon Research Conference (1995) and an IUPAC international symposium on Natural Products Chemistry (1996). He was a founding Associate Editor and is now member of the Editorial Board of *Organic Process Research and Development*. He enjoys golf, writing poetry, and studying philosophy.

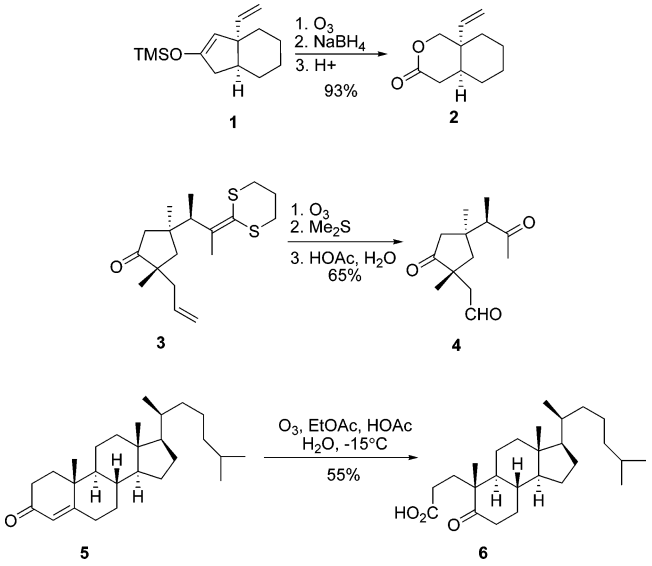
Scheme 1. Schreiber's Approach to Ozonolysis of Cycloalkenes



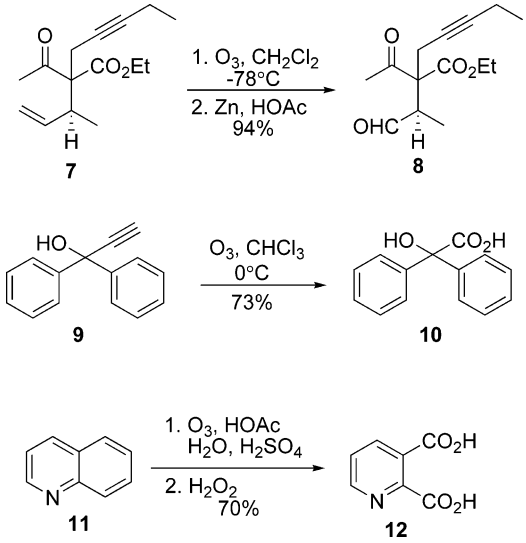
Ozonation of alkynes typically furnishes carboxylic acids in methanol. Alkene **7** can be selectively cleaved in the presence of alkynes to provide aldehyde **8** as shown in Scheme 3.[18a] Aromatic rings are more stable to ozone, and a preferential reaction takes place with alkynes to provide the carboxylic acid **10**.[18b] Heteroaromatic systems such as quinoline **11** are ozonized to the corresponding diacid **12** under the rigorous conditions of acetic acid, excess ozone, and hydrogen peroxide.[19] In this particular case, ozone did not react with the quinoline nitrogen to produce the corresponding *N*-oxide. Additional heteroatom ozonolysis reactions include the conversion of phosphines to phosphine oxides,[20] organic sulfides to sulfoxides,[21] and selenides to selenoxides.[22]

The use of aluminum chloride or boron trifluoride[23] as additives in ozonolysis reactions can enhance the nucleophilicity of unsaturated groups. However, as shown in Scheme 4, the opposite effect is realized by the addition of pyridine to the reaction of alkene **13**, which results in higher yields and regioselectivity to provide aldehyde **14**.[24]

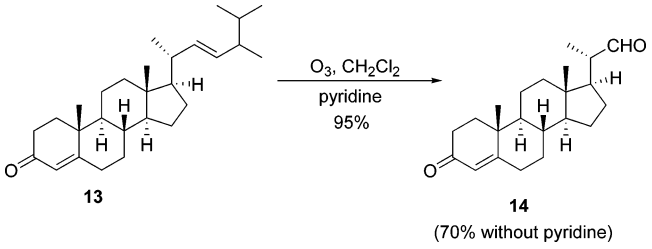
Scheme 2. Ozonolysis Extensions



Scheme 3. Ozonolysis of Unsaturated Systems



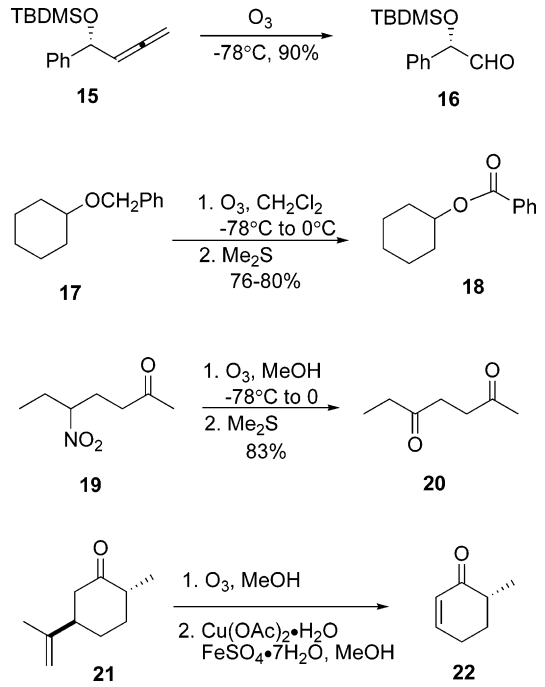
Scheme 4. Regioselective Ozonolysis



Ozonolysis reactions can be extended to oxidative transformations to provide basic functional group preparation. Allenes such as **15** are susceptible to ozonolysis to provide aldehydes **16**[25] as well as ozonation of benzyl ether **17** to afford a high yield of benzoate ester **18**.[26] Cleavage of nitronate anion **19** with ozone affords the corresponding ketone **20**.[27] Finally, a simple chemical transformation for converting alkene **21** to enone **22** has been accomplished by an ozonolysis reaction (Scheme 5).[28]

This introduction provides a survey of where ozone has been used to conduct a wide variety of chemical transformations. This review aims to provide some recent examples of ozonolysis chemistry used in total synthesis including the

Scheme 5. Ozonolysis Extensions



preparation of biologically active molecules, followed by examples of known industrial-scale processes used in pharmaceutical synthesis. After a review of the literature, these authors found that most cited ozonolysis reactions are conducted on a small scale and involve typical alkene cleavage reactions. Many large-scale ozone reactions conducted at chemical and pharmaceutical companies are not published for proprietary reasons. Additional references employing ozonolysis reactions can be found in the References described for each compound synthesized.[29]

2. Ozonolysis in Total Synthesis

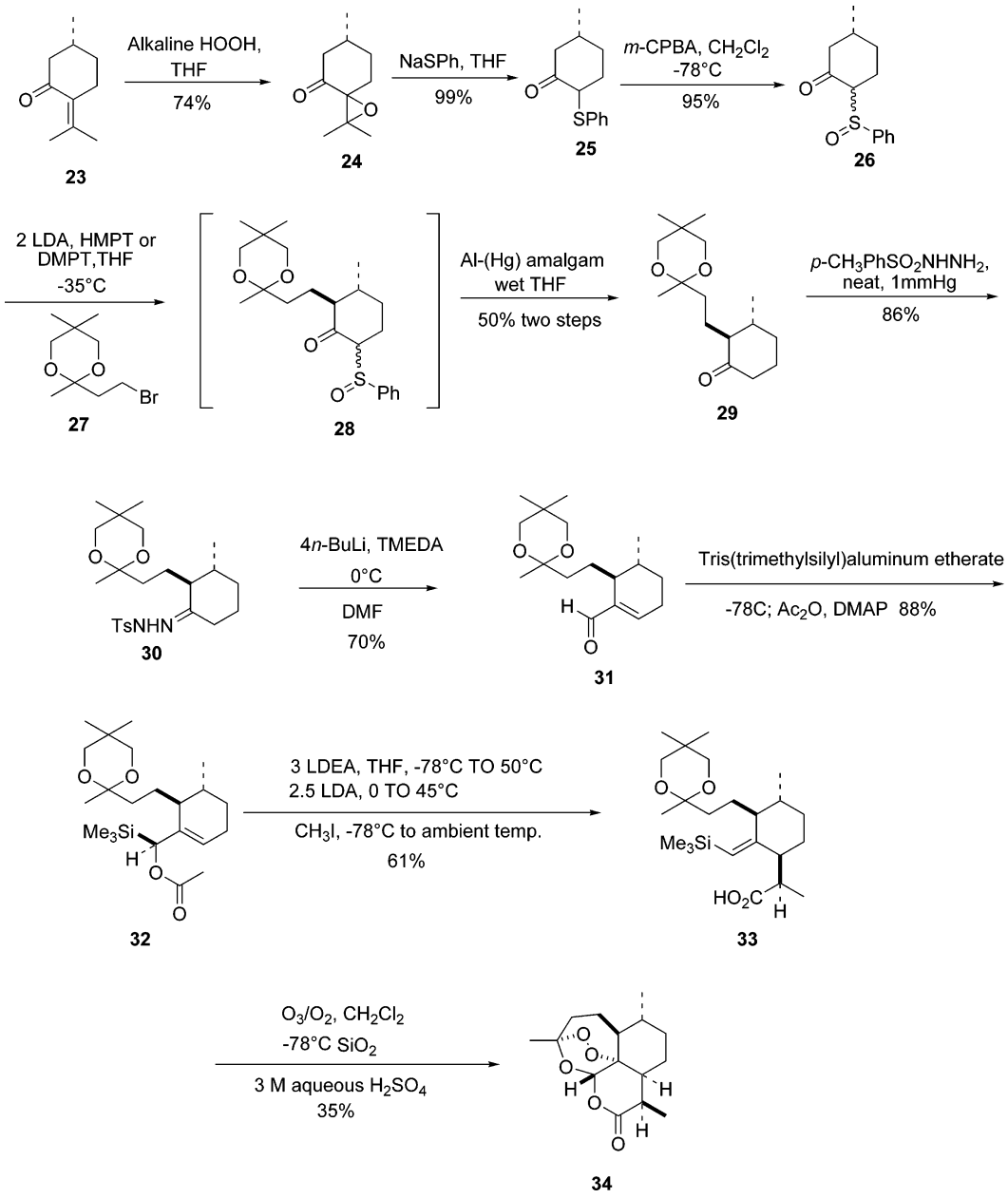
2.1. (+)-Artemisinin

Extracted from the plant *Artemesia annua* L., (+)-artemisinin has emerged as a potent antimalarial stable peroxide useful against resistant strains of *Plasmodium falciparum*.[30] (+)-Artemisinin contains an "endoperoxide bridge", which reacts with iron atoms to form free radicals. Artemisinin becomes toxic to malaria parasites because it reacts with the high iron content of the parasites, generating free radicals and leading to the damage of the parasite. Recent studies also suggest that artemisinin is effective at treating various forms of cancer such as leukemia and breast cancer.[31] The total synthesis[32] of artemisinin has been reported in addition to congener synthesis over recent years.[33] Avery and co-workers describe a 10-step stereoselective total synthesis starting with (*R*)-(+)-pulegone **23**, which employs an abnormal ozonolysis process in the last step to provide (+)-artemisinin **34**.[34,35]

(*R*)-(+)-Pulegone **23** was epoxidized with alkaline hydrogen peroxide to pulegone epoxide **24** in 74% yield (Scheme 6). Thiophenoxide ring opening of **24** and loss of acetone afforded the thiophenyl ketone **25** in regiospecific fashion in high yield.

Oxidation of **25** with *m*-chloroperoxybenzoic acid furnished sulfoxide **26** in 95% yield. Alkylation of ketone **26** with LDA followed by 2-(2-bromoethyl)-2,5,5-trimethyl-1,3-dioxane **27** provided transient intermediate **28** that was desulfurized with aluminum amalgam to afford the desired

Scheme 6. Avery's Approach to (+)-Artemisinin

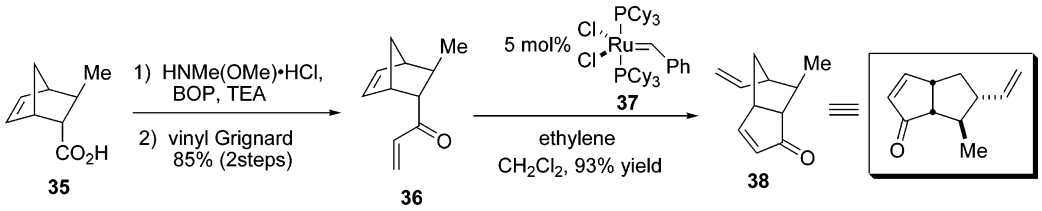


ketone **29** in 50% yield. The reaction of ketone **29** with *p*-toluenesulfonohydrazide provided the corresponding hydrazone **30** in 86% yield. Subsequent treatment of hydrazone **30** with 4 equiv of *n*-butyllithium and *N*,*N*-dimethylformamide (DMF) provided the regiochemically pure unsaturated aldehyde **31** in 70% yield. Diastereomer **32** was furnished by the reaction of aldehyde **31** with tris(trimethylsilyl)-aluminum etherate in 88% yield. A convenient one pot approach to vinyl silane **33** was developed by reacting **32** with 3 equiv of lithium diethylamide followed by 2.5 equiv of LDA. The resultant dianion of **32** was quenched with methyl iodide to afford acid **33** in 61% yield.

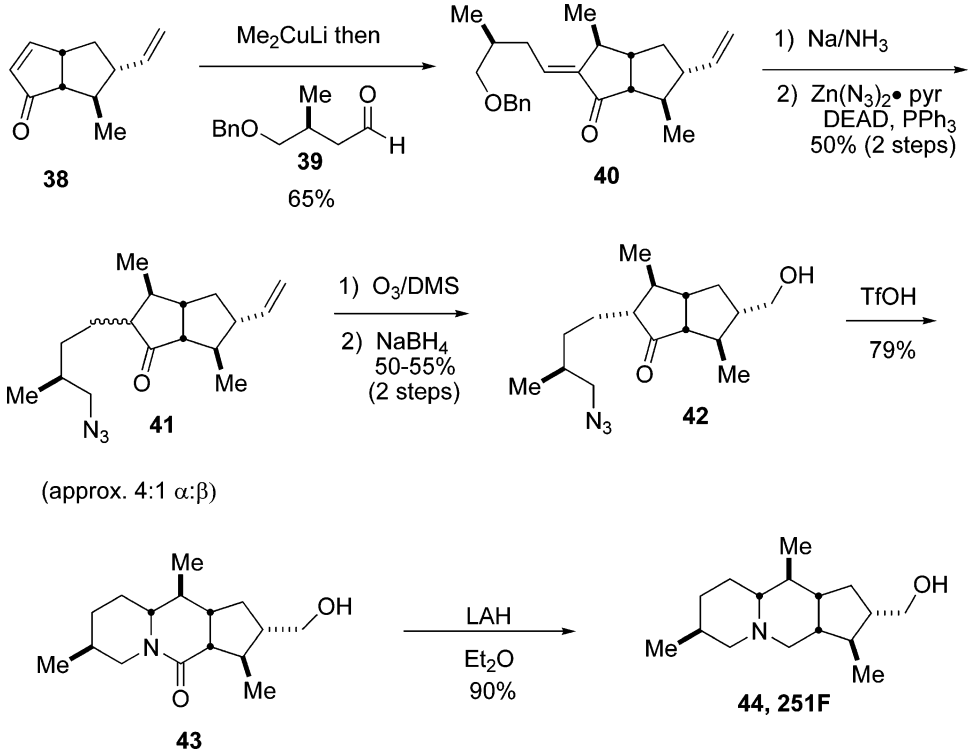
The final strategy employs an abnormal ozonolysis process in the last step to provide (+)-artemisinin **34**. The remarkable sequence from **33** to **34** can be explained by protonation of the middle oxygen (O2) of the primary ozonide and heterolytically breaking the O1−O2 bond during the migration of the silicon from carbon to O1 in a Beckman-type anionotropic shift. Similar ozonolysis results are discussed by Büchi and Wüest,[34b] where a chemiluminescent dioxetane is isolated in low yield (15%). Such a dioxetane (structure 3 in ref 33a) could form in this case after the migration of the tetramethylsilane (TMS) group, or the peroxy aldehyde could proceed directly to the product after nucleophilic cleavage of the TMS group. Another case of silicon involved in a Beckman rearrangement or fragmentation was observed by Hudrlik et al.[34c]

Ozonolysis of acid **33** in dichloromethane at −78 °C followed by the addition of aqueous sulfuric acid and silica gel furnished (+)-artemisinin **34** in 33−39% yield. Dichloromethane proved to be the superior solvent for the reaction. Methanol destroyed the tetracycle via lactone ring methanolysis, and other solvents surveyed (hexane and ethyl acetate) led to lower yields compared to dichloromethane. Efforts to maintain oxidative reaction conditions were investigated with additives such as *tert*-butylhydroperoxide and *tert*-butylperoxide, but this proved unsuccessful. The addition of *tert*-butylhydroxytoluene resulted in the highest yield of 35% of (+)-artemisinin. After the ozonolysis sequence was completed, the treatment with sulfuric acid provided the cyclization reaction to (+)-artemisinin **34** in 35% yield.

Scheme 7. Construction of the Key Bicyclic Enone



Scheme 8. Aúbe's Approach to Indolizidine 251F



2.2. Indolizidine 251F

Indolizidine 251F is a natural product derived from skin extracts of the dendrobatid frog species *Minyobates bombetes*. The skin exudate of the Colombian frog *M. bombetes* causes severe locomotor difficulties, muscle spasms, and convulsions upon injection in mice. The major component of the alkaloid mixture is 251F. As a class, these alkaloids include numerous biological actives of which batrachotoxin and epibatidine are known examples.[36] The activity of 251F has not been established, and the challenge of the total synthesis rests with forming three fused rings and seven stereogenic centers. The first total synthesis of 251F was reported by Taber and You[37] employing a diastereoselective rhodium-catalyzed synthesis of a key cyclopentane intermediate. Aúbe and co-workers report an asymmetric synthesis of 251F employing an intramolecular Schmidt reaction as well as an asymmetric Diels−Alder reaction.[38] Ozonolysis of an advanced intermediate eventually would provide quantities of 251F for biological screening.

As shown in Scheme 7, the Diels−Alder adduct **35** was converted to vinyl ketone **36** via the Weinreb amide[39] in 85% yield. Treating **36** with Grubb's catalyst **37** in an atmosphere of ethylene provided the corresponding bicyclic enone **38** in 93% yield.

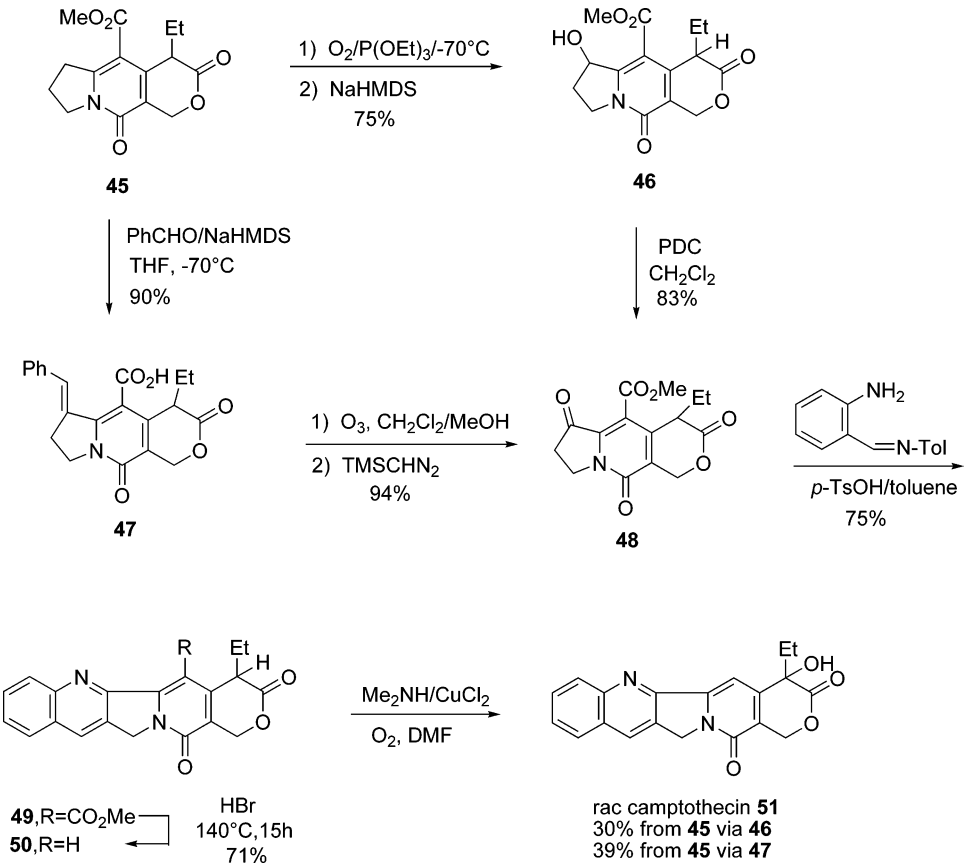
Derivatization of enone **38** was required to provide the intramolecular Schmidt precursor. Treatment of **38** with lithium dimethyl cuprate followed by the addition of aldehyde **39** resulted in the formation of enone **40** in 65% yield. The desired *exo* stereochemistry of the methyl substituent was effected because of cuprate addition occurring from the exposed face of **38**. The treatment of **40** with Na/NH_3 facilitated the reduction of the enone and cleavage of the benzyl ether moiety. The 4:1 mixture of inseparable diastereomers was carried directly into conversion of the alcohol into its azide derivative **42** using a modified Mitsunobu reaction.[40]

When standard conditions[41] for carrying out the intramolecular Schmidt reaction were tried on **41**, only degradative products were observed. To circumvent this problem, ozonolysis was carried out on **41** followed by the reduction with sodium borohydride. The azide functionality survived the ozone treatment. Column chromatography was then used to separate the mixture of diastereomers to produce alcohol **42**. The intramolecular Schmidt reaction was then conducted by treatment of **42** with trifluoromethanesulfonic acid to furnish lactam **43** as a single diastereomer in 79% yield. The total synthesis of alkaloid 251F was completed by the reaction of **43** with lithium aluminum hydride to provide **44** in 86−100% yield (Scheme 8). Spectral characteristics were consistent with literature values, and optical rotation data indicated an enantiomeric excess of 93%.

2.3. D,L-Camptothecin

Renewed interest in synthesizing camptothecin and its active analogues has been triggered by findings of its high

Scheme 9. Danishefsky's Approach to D,L-Camptothecin



antitumor activity in various cell lines and animal screens.[42] While camptothecin is a difficult alkaloid to isolate, its analogues are synthesized in good yields from the natural product.[43] Danishefsky and co-workers re-evaluated the problem and explored the chemistry to provide a two pronged approach to an inexpensive and decent yielding route to D,L-camptothecin.[44]

Shown in Scheme 9, the readily available tricyclic ester **45** was converted to **48** using two routes. In the first sequence, **45** was reacted with sodium hexamethyldisilazide and benzaldehyde to afford the benzylidene acid **47** in 90% yield. Ozonolysis of **47** followed by esterification provided **48** in 94% yield. The complementary route involved the reaction of tricylic **45** with oxygen and triethylphosphite to provide a 75% yield of diastereomers **46**. Oxidation with pyridinium dichromate afforded the corresponding ketone **48** in 83% yield.

The reaction of ester **48** with the Schiff base shown via a Friedlander condensation afforded the pentacyclic compound **49**. Heating **49** with HBr provided D,L-desoxycamptothecin **50** in 71% yield. Hydroxylation of **50** with oxygen, Me_2-NH, and $CuCl_2$ [45] gave the title compound camptothecin **51** in 91% yield.

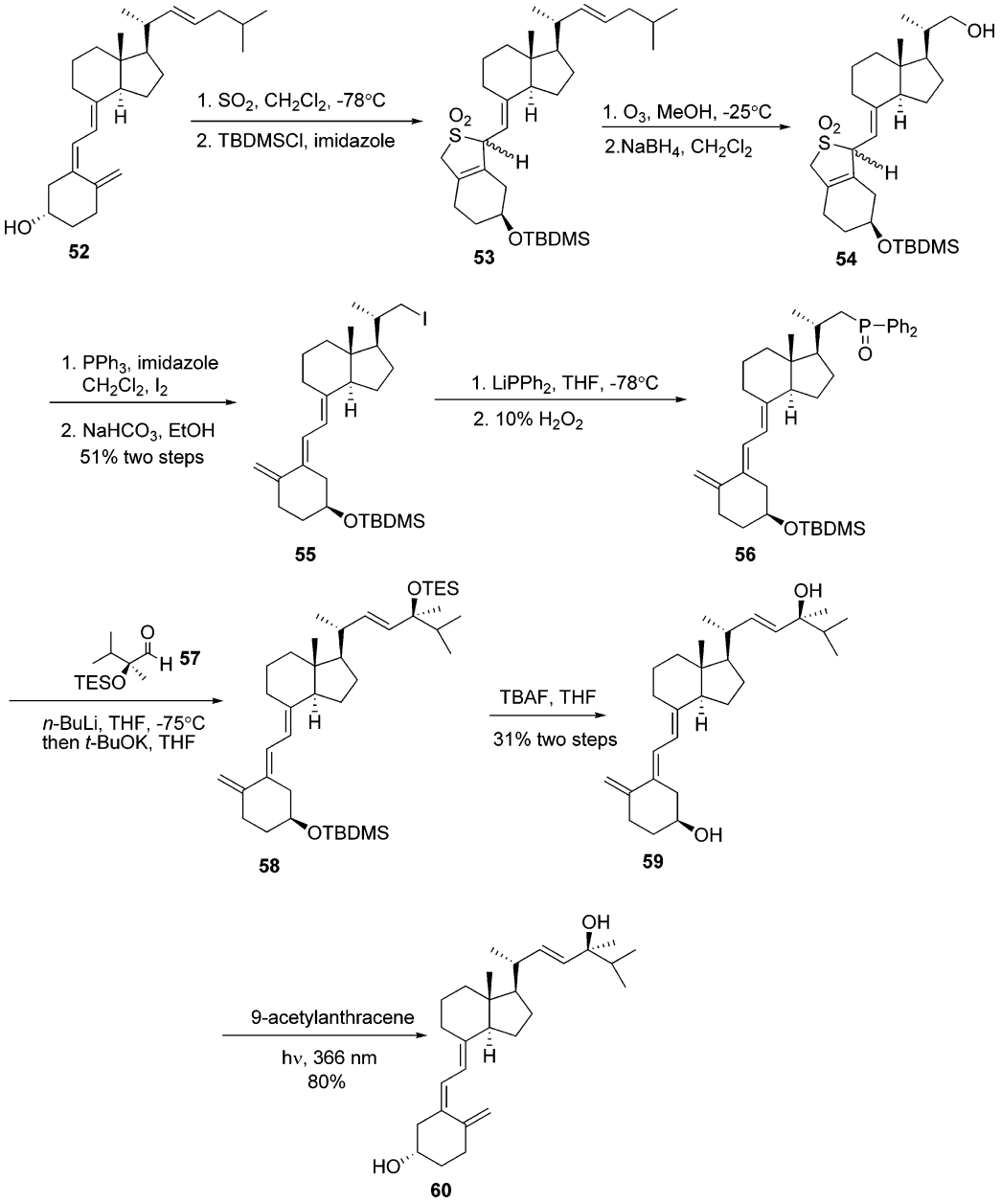
2.4. 24(S)-Hydroxyvitamin D_2

Vitamin D analogues have long been regarded as important compounds in bone and mineral metabolism. Prodrugs of vitamin D compounds are often sought because they are biologically inactive as administered but eventually are metabolized to active substrates in vivo. Ergocalciferol has been a starting material for the synthesis of numerous analogues of vitamin D, including the vitamin D_2 metabolite, 24(S)-hydroxyvitamin D_2. The first stereospecific synthesis of this metabolite was carried out by Meckler et al.[46] This metabolite acts as a prodrug for 1α,24(S)-dihydroxyvitamin D_2, which is under development for treatment of diseases characterized by cellular hyperproliferation.

As shown in Scheme 10, ergocalciferol **52** was reacted with SO_2 to provide an unstable mixture of C-6/C-19 epimeric SO_2 adducts, followed by silylation to afford the protected mixture **53**. Ozonolysis of **53** was conducted in a solution of methanol/dichloromethane (1:3) at −25 °C, but the yields were inconsistent. Tsuji and Ishikawa have reported the catalytic use of additives ($CaCl_2$, $MgCl_2$, $AlCl_3$, $ZnCl_2$, or $TiCl_4$) to act as an ozonolysis stabilizer.[47] Thus, 1 equiv each of sodium acetate and acetic acid were added to the reaction mixture, which resulted in consistent reaction yields. It is not known if this is due to a stabilizing salt effect or to the sodium acetate/acetic acid buffering of the reaction. Reductive workup of the reaction mixture with sodium borohydride afforded the crude alcohol **54**, which was then iodinated. The diastereomeric mixture of primary iodides was subjected to thermal elimination of SO_2 in refluxing ethanol to provide iodide **55** in 51% yield over six steps. Iodide **55** was treated with lithium diphenylphosphide followed by oxidative workup with hydrogen peroxide to furnish phosphine oxide **56**. Optically active aldehyde **57** and phosphine oxide **56** were coupled to afford the desired olefin **58**.

Deprotection of the hydroxyl groups using tetrabutylammonium fluoride in tetrahydrofuran (THF) provided the *trans*-24(S)-hydroxyvitamin D_2 **59**. Photoisomerization to the *cis*-24(S)-hydroxyvitamin D_2 was effected in methanol in the presence of the photosensitizer, 9-acetylanthracene, to provide an 80% yield of the title compound **60**.

Scheme 10. Meckler's Approach to 24(S)-Hydroxyvitamin D_2



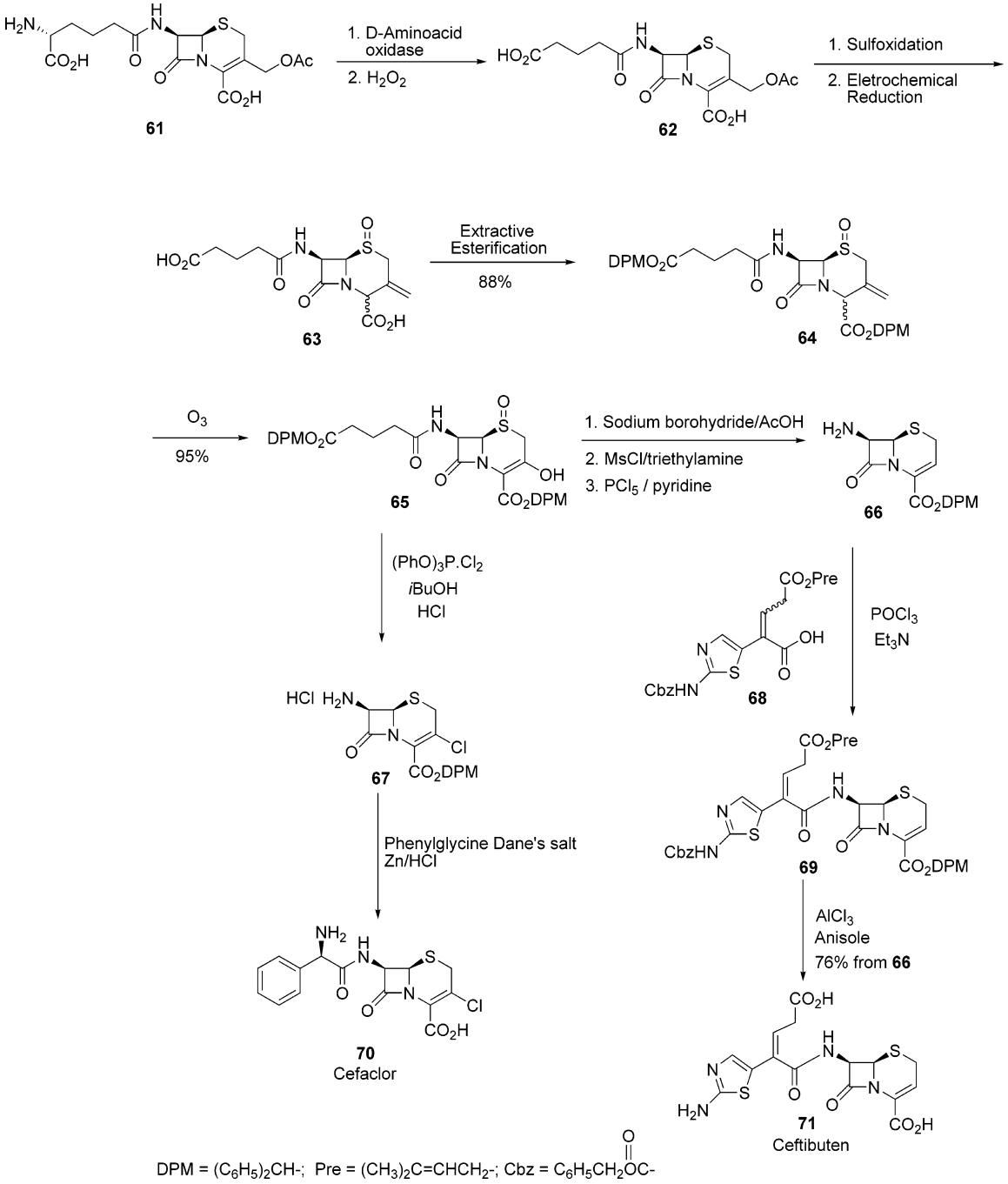
3. Industrial-Scale Ozonolysis

3.1. Ceftibuten and Cefaclor

Ceftibuten is a third-generation oral cephalosporin, has excellent Gram-negative activity, and possesses a high degree of β-lactamase stability. Current clinical trials indicate that ceftibuten may be effective in the treatment of acute otitis media, streptococcal pharyngitis, acute exacerbations of chronic bronchitis, and urinary tract infections. Cefaclor is used to treat certain infections caused by bacteria, such as pneumonia and infections of the ears, lungs, throat, urinary tract, and skin. Cefaclor and ceftibuten are in a class of medications called cephalosporin antibiotics. Earlier Shionogi Research Laboratories described a synthesis on industrial scale for producing ceftibuten.[11] In an effort to develop commercial routes to these valuable compounds, researchers at the Schering Plough Research Institute utilized an ozonolysis reaction to provide a key intermediate used to produce ceftibuten and cefaclor.[48]

As shown in Scheme 11, cephalosporin C **61** was enzymatically transformed to the carboxylic acid **62** followed by electrochemical reduction to afford sulfoxide **63**. An extractive esterification using diphenyldiazomethane in a biphasic aqueous dichloromethane solution resulted in the formation of ester **64** in 88% yield. Ozonolysis of 3-exomethylenecephams in the sulfoxide or sulfide form have been previously described in the literature.[49] After an aqueous washing procedure to remove monoacid impurities, the resulting methanol solution of **64** was ozonized at −55 °C for 4−6 h and then quenched with trimethyl phosphite to provide a nearly quantitative yield of **65**. Additional development of this step showed that the ozonolysis of the sulfoxide form was superior to ozonolysis of the sulfide form of **64**. It was believed that the sulfoxide form was more stable based on a 95% yield, whereas ozonolysis of the sulfide of **64** led to lower yields. Intermediate **65** was reacted in a two-step process first to provide amine **67** and then reacted with phenylglycine to afford cefaclor **70**. Multiple routes were

Scheme 11. Ceftibuten and Cefaclor Synthetic Route



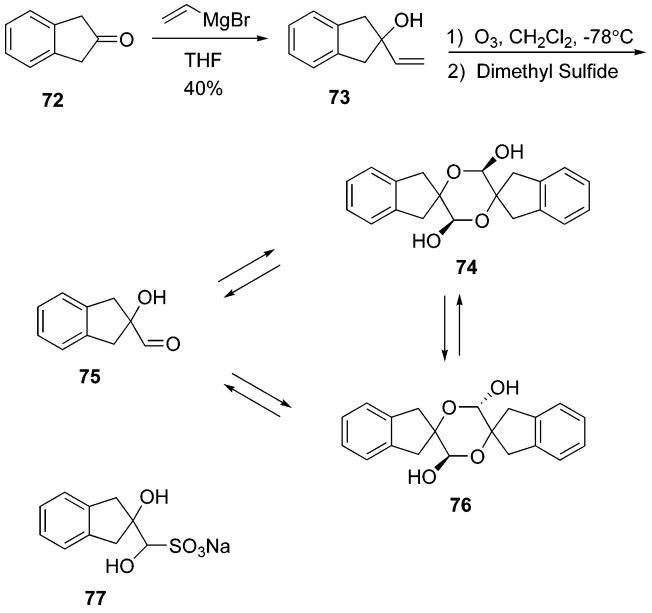
investigated to transform sulfoxide **65** to **66**. A three-step sequence was carried out through a series of reactions to afford the amine **66**. The coupling reaction of amine **66** with acid **68** in the presence of phosphorus oxychloride furnished amide **69**. Subsequent deprotection of **69** with aluminum chloride in anisole provided ceftibuten **71**. Further purification provided an overall yield of 76% from **66** to **71**.

3.2. 2-Hydroxyindan-2-carboxaldehyde

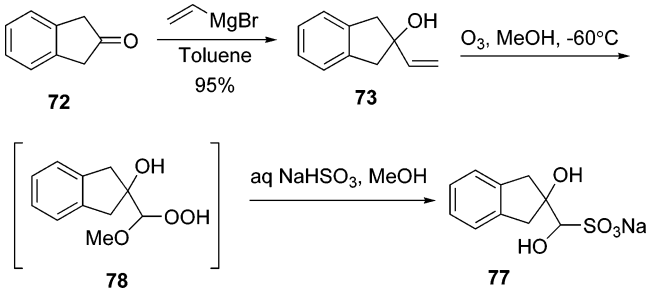
Ozonolysis reactions typically are limited to small-scale reactions. However, pharmaceutical and chemical companies have been able to safely execute large-scale ozonolysis processes. After careful assessment of the safety and calorimetry data, chemists at Pfizer prepared a bisulfite adduct of 2-hydroxyindan-2-carboxaldehyde that was used as a surrogate aldehyde in a reductive amination reaction.[50] Shown in Scheme 12, the reaction of 2-indanone **72** with vinylmagnesium bromide in THF afforded the tertiary allylic alcohol **73** in 40% yield. The low yield can be attributed to competitive enolization of the starting ketone.

Ozonolysis of the vinyl moiety in dichloromethane at −78 °C followed by reductive workup with dimethyl sulfide afforded a complex mixture of products represented by dimers **74** and **76**, in which the monomeric aldehyde **75** was a minor component. The product mixture was then subjected to reductive amination conditions and acted as if a free aldehyde was present. The interpretation of this result suggested that the α-hydroxyaldehyde forms an equilibrium mixture of dimers and oligomers that condense with the secondary amine to form the corresponding iminium species for subsequent reductive amination. This chemistry could be carried out on a laboratory scale but was thought likely

Scheme 12. Pfizer's First Route toward Key Bicyclic Aldedyde 75



Scheme 13. Preparation of Bisulfite by Large-Scale Ozonolysis

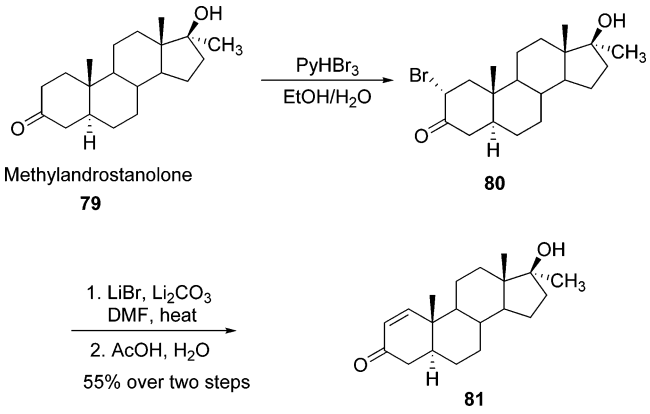


to cause concern on scale-up to multi-kilogram quantities. The instability of aldehyde **75** suggested the idea of a bisulfite adduct **77** as an intermediate precursor for the reductive amination. A more efficient process was developed to provide the required bisulfite adduct **77** directly from the ozonolysis sequence as shown in Scheme 13.

The first improvement was realized by using toluene instead of THF for the Grignard addition of vinylmagnesium bromide to 2-indanone **72**. This increased the yield to over 95%, and the crude alcohol **73** could be employed directly in the ozonolysis step. The alcohol **73** was cooled to −60 °C in methanol to trap the carbonyl oxide as the methoxyhydroperoxide **78**.[51] Reduction of the hydroperoxide could be effected with dimethyl sulfide, potassium iodide, or sodium bisulfite. After the reaction mixture was added to an aqueous slurry of sodium bisulfite, the exotherm could be controlled effectively during the reduction sequence. Warming the slurry to 60 °C completed the bisulfite transformation to **77**. Bisulfite adduct **77** was isolated in 61% overall yield from 2-indanone.

Prior to scale-up, it was necessary to investigate several parameters of the reaction to provide a safe process to perform in the pilot plant. Analysis of the reaction by RC-1 calorimetry suggested a ΔH_{obs} of −535 kJ/mol, which corresponds to an adiabatic heat rise of 170 °C. This value essentially indicates the total heat that would need to be removed by the reactor jacket. Considering that the reaction rate was dose-controlled, the rate of addition of ozone could be stopped if the reaction temperature approached intolerable limits.

Scheme 14. Preparation of Key Intermediate 81



Differential scanning calorimetry also indicated that the reaction mixture possessed an exothermic event occurring near 45 °C and expelling 404 J/g of energy. This suggested a window greater than 100 °C for the reaction at −65 °C; therefore, it was deemed safe for quenching at 0 °C. The thermal stability results, coupled with the dose rate control data, provided confidence that the reaction could be performed safely on a kilogram scale.

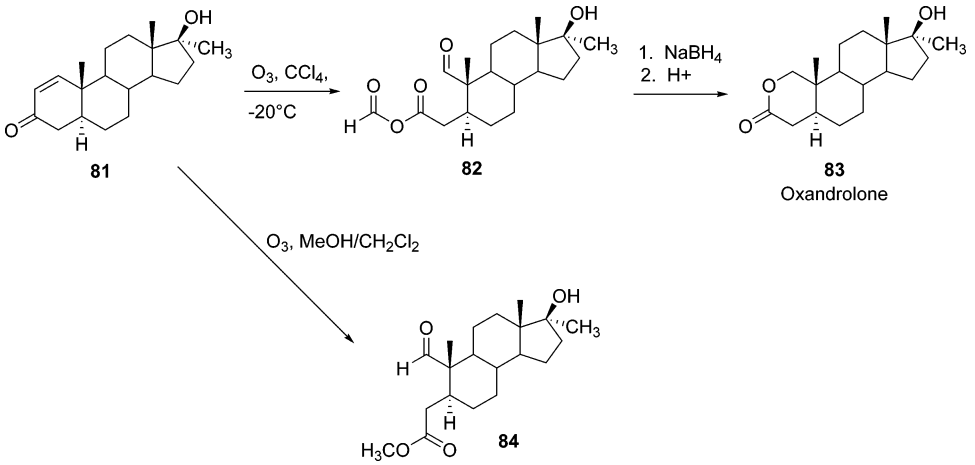
Solvent flammability was also a primary concern in addition to gases forming in the headspace of the reactor, which could lead to an explosive situation. The solution to this problem was diluting the ozone with a higher flow of an inert gas (nitrogen) in an effort to lower the risk of explosion. Three engineering scenarios were mapped out considering the moles of ozone produced per hour and rate of flow of ozone and inert gas. The safest option provided a process that kept both methanol and oxygen/ozone below the lower flammability limits and minimum oxygen concentration. With the safety controls in place and an appropriately engineered setup to dissipate the flammable gases, the ozonolysis reaction was scaled to the 3 kg level. The crude alcohol **73** was diluted with methanol and cooled to −60 °C, and ozone gas was added over 19 h to complete the reaction. After the reaction was warmed to 0 °C, it was transferred to the aqueous sodium bisulfite quench vessel. Warming the mixture to 60 °C completed the synthesis to afford bisulfite adduct **77**. This assessment of the safety of an ozonolysis reaction demonstrates the ability to safely scale ozonolysis procedures in the preparation of pharmaceutical intermediates.

3.3. Oxandrolone

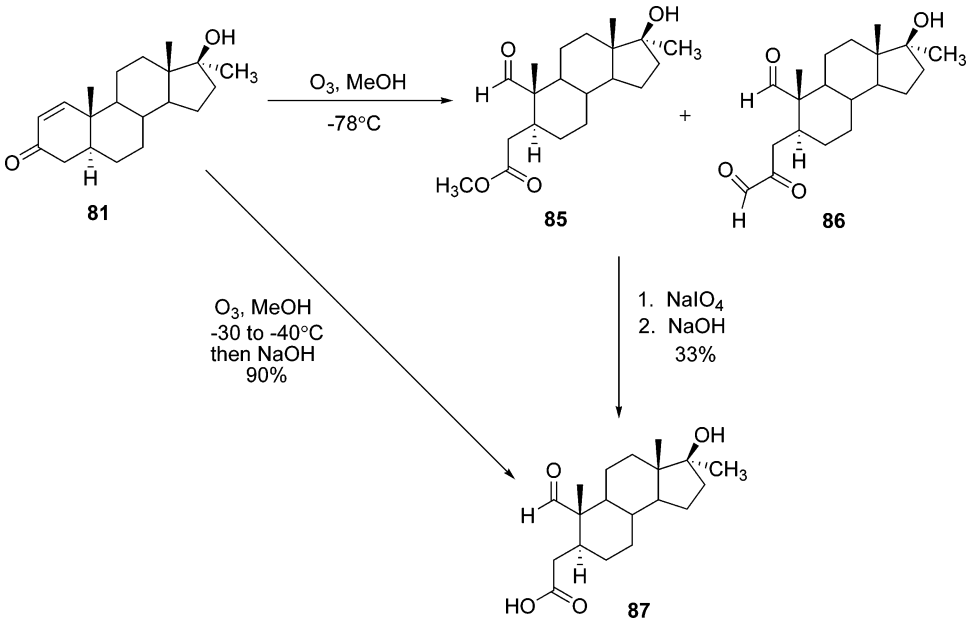
Oxandrolone is an anabolic steroid used to promote weight gain following extensive surgery, chronic infection, or severe trauma and in other cases that result in inadequate weight gain or maintenance. Oxandrolone is also used to decrease muscle loss caused by the treatment with corticosteroids and to reduce bone pain associated with osteoporosis. A four-step process employing a large-scale ozonolysis sequence is described below by Cedarburg Pharmaceuticals, Inc. to provide kilogram quantities of oxandrolone.[52]

Methylandrostanolone **79** was reacted with pyridinium tribromide in ethanol to provide the α-bromo ketone **80**. Elimination of HBr was facilitated with lithium bromide in DMF to provide the corresponding ketone **81** in 55% yield over two steps (Scheme 14).

Scheme 15. Initial Ozonolysis Studies toward Oxandrolone



Scheme 16. Modified Ozonolysis Approach

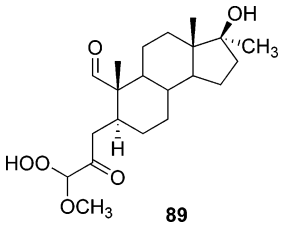


Extensive studies on the ozonolysis step were carried out to find a safe and scaleable process. A thorough search of the patent literature revealed that this approach was used by a group at Searle in 1963 to synthesize oxandrolone.[53] The Searle patent describes the ozonolysis of **81** in CCl_4 to give the mixed anhydride **82**, which was then converted directly to oxandrolone **83** after the reduction of the aldehyde and cyclization (Scheme 15). Alternatively, performing the ozonolysis step in a mixture of methanol and methylene chloride provided methyl ester **84**. The use of the carcinogenic CCl_4 was not suitable for large-scale production. Moreover, in these processes, the potentially dangerous ozonide or peroxide intermediates are decomposed thermally.

Initially, the ozonolysis was performed in methanol, and a traditional dimethyl sulfide workup was employed. Under these conditions, several products were formed by thin-layer chromatography (TLC). The major components presumably were the keto-dialdehyde **86** and the desired diester **85** (Scheme 16). Further oxidation of this mixture with $NaIO_4$ gave mainly the acid **87** by TLC analysis. Other typical ozonide cleaving agents [$P(OCH_3)_3$, PPh_3, Zn, etc.] were not evaluated.

The two-step oxidation method was moderately successful but provided a product that contained multiple unidentified impurities. The procedure described by Bailey for workup of ozonolysis products using aqueous sodium hydroxide[54] was investigated. This procedure eliminated the need for odiferous cleavage agents and allowed for the purification of the acid during the workup via standard acid−base chemistry. Further oxidation with $NaIO_4$ was unnecessary, because the sodium salt of the acid was the initial product formed after base quenching. The ozonolysis of **81** in methanol from −30 to − 40 °C, followed by quenching with an aqueous solution of sodium hydroxide at ca. −15 °C, gave the desired acid **87** in 90% yield with a purity of 99% by high-performance liquid chromatography (HPLC) (area %) as shown in Scheme 16. The success of this reaction allowed the desired oxidation to take place without using OsO_4 and $Pb(OAc)_4$, which are not only highly toxic but also present a disposal problem.

The temperature effects of the ozonolysis reaction were examined, and it was found that performing the reaction at temperatures from −50 to −30 °C gave high-quality **87**, while running the reaction at temperatures from 0 to −20 °C gave a lower quality product. However, because of cooling limitations at the reactor scale, the reaction was run from −15 to 5 °C. Recrystallization of acid **87** provided an acceptable quality product.



89

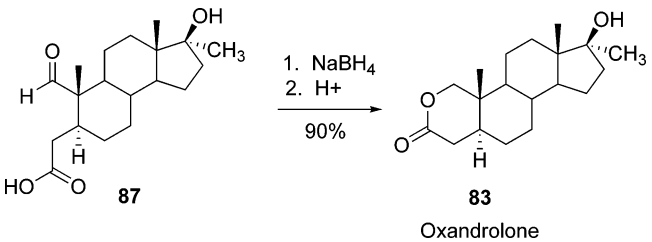
Figure 1. Intermediate **89**.

The effects of different solvents in ethanol led to multiple product formation. Reactions in ethyl acetate, CH_2Cl_2, and isopropanol led to slow conversions presumably because of the insolubility of **82** in these solvents. The reaction in a mixed solvent system of CH_2Cl_2/acetic acid led to multiple product formation as well. The current operating procedure for converting **81** to **87** has been scaled up to produce in excess of 14 kg of acid **87**. Ozone is sparged into a methanolic solution of **81** until the reaction was deemed complete by quantitative TLC analysis. Monitoring the reaction by HPLC is complicated by the fact that the product does not contain a strong chromophore for UV analysis. On a 14 kg scale, the reaction was complete ($\leq$0.5% **81)** after approximately 24 h. Extended treatment with ozone leads to the formation of a more polar compound, soluble in aqueous NaOH. This compound was characterized as the acid **87** by nuclear magnetic resonance (NMR) spectroscopy and can be controlled by performing the workup immediately after the ozonolysis is complete.

It is assumed that the intermediate formed in the ozonolysis is the hydroperoxide **89** (Figure 1). Hydroperoxides in general have been known to be thermally unstable and thus potentially dangerous intermediates. An advanced reactive system screening tool (ARSST) experiment was conducted on a methanolic solution of **89** to address this issue. When a sample of the reaction intermediate was heated from 25 to 100 °C in a sealed vessel at a rate of 2 °C/min, there was no temperature increase above the input temperature. The internal pressure also rose slightly because of the heating of methanol above its boiling point. It was determined that this particular hydroperoxide was intrinsically safe at the proposed ozonolysis-operating temperatures.

After the workup, the crude acid **87** was recrystallized from methanol to afford 60−70% consistent yields of pure product. The final step involved the reduction of acid **87** followed by acid-catalyzed cyclization to provide oxandrolone **83** in 90% yield (Scheme 17).

Scheme 17



87 83

Oxandrolone

4. Conclusion

Ozone continues to be a clean and efficient reagent for organic chemists in the laboratory and beyond. The examples presented herein highlight the successful use of ozone in both academic synthesis and industrial chemical processes. From small to large scale, researchers and process-development chemists have safely implemented ozonolysis reactions in their efforts to bring interesting new products to the pharmaceutical and fine-chemical market.

5. Acknowledgment

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