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2007 American Chemical Society Team Innovation Award Address

Linezolid (ZYVOX), the First Member of a Completely New Class of Antibacterial Agents for Treatment of Serious Gram-Positive Infections

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Of the 35 million people admitted every year to the hospital in the U.S., an estimated 2 million patients find that they have contracted a hospital-acquired or so-called nosocomial infection, a serious complication that few would ever have anticipated. It is estimated that nosocomial infections lead to 90 000 deaths per year in the U.S. and that 70% of these infections are caused by bacterial pathogens that have become resistant to one or more antibiotics.[1] The majority of such hospital-acquired infections are caused by Gram-positive pathogens, among which the most problematic are methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis*, vancomycin-resistant *Enterococcus faecium* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP).[a] The degree of the global medical community's concern over such resistance problems becomes readily apparent when one considers the dramatic increase in the incidence of MRSA over the past decade and a half. Surveillance data reported by the National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC) indicate that MRSA incidence among *S. aureus* isolates collected from patients in U.S. intensive care units (ICUs) rose from a 1974 level of only 2% to 22% in 1995 and to 64% in 2004.[2]

The emergence of serious VRE infections has likewise become very worrisome, as prior to the late 1980s, strains of normal intestinal flora such as *Enterococcus faecalis* or *E. faecium* would rarely have been found to be the cause of serious complicated invasive infections. But an apparent unintended consequence of the more frequent use of vancomycin for MRSA infections, or prophylactic treatment in certain surgical procedures, has been the emergence of high-level vancomycin resistant enterococci in hospitals worldwide.[3] The first reports emanated from Europe in 1988,[4] and soon thereafter many additional cases were found in various U.S. hospitals. Twenty years later we now see a highly predominant incidence of vancomycin-resistance in *E. faecium* strains collected from ICUs in the U.S.,[5] while most *E. faecalis* strains are still vancomycin sensitive. These difficult-to-treat, multidrug-resistant VRE pathogens are associated with high mortality rates, as they cause very problematic and sometimes untreatable infections that commonly afflict the immunocompromised.

Streptococcus pneumoniae is a more common pathogen encountered in the community setting. As implied by the species name, it is implicated in many of the bacterial pneumonia cases that annually cause 40 000 deaths in this country. It is remarkable that for nearly 50 years penicillin proved to be quite effective against most pneumococci, as there was only an extremely low level (<0.1%) of penicillin resistance during those decades. However, beginning in 1988, a surge in the extent of penicillin resistance in the pneumococci began to be observed,

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[a] Abbreviations: aa-tRNA, aminoacyl-tRNA; CAP, community-acquired pneumonia; CYP_{450}, cytochrome P450; ELF, epithelial lining fluid; ICUs, intensive care units; MDR, multidrug resistant; MIC, minimum inhibitory concentration; MOA, mechanism of action; MRSA, methicillin-resistant *Staphylococcus aureus*; NDA, new drug application; NOAEL, no observed adverse effect level; PRSP, penicillin-resistant *Streptococcus pneumoniae*; rRNA, ribosomal RNA; SSTI, skin and soft tissue infections; STR, structure−toxicity relationship; VRE, vancomycin-resistant *Enterococcus*.

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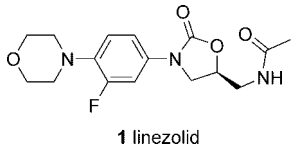
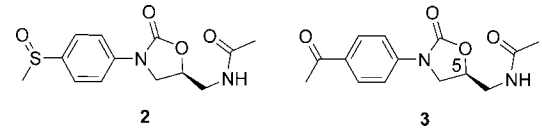


1 linezolid

Figure 1. Structure of linezolid, the first approved oxazolidinone antibacterial agent.



2 **3**

Figure 2. Structures of DuPont oxazolidinone leads.

and by 1999, 25% of the U.S. isolates surveyed were penicillin resistant.[6] Furthermore, many of these PRSP strains also exhibit high-level resistance to alternative antibiotics such as the cephalosporins, tetracyclines, and macrolides.

These and other equally disconcerting multidrug-resistant strains prevalent among Gram-negative species like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have led many to ask the question: how can we effectively address these serious threats to public health? Infectious diseases experts, the pharmaceutical industry, and others in the public health infrastructure have repeatedly called for the responsible use of antibiotics. Significant focus has been directed toward educating the public, with campaigns to teach that antibiotics do not have utility against viral respiratory tract infections, that patients who pressure their physicians to immediately prescribe antibiotics may be contributing to the resistance problem by adversely disrupting normal bacterial flora, and that it is important for patients to complete their full course of antibiotic therapy. Measures have also targeted medical practitioners to ensure the susceptibility of the infectious organism to the antibiotic they prescribe. These efforts notwithstanding, a fundamental and likely insurmountable challenge remains: that our use of antibiotics drives a cycle of selection for resistant bacterial populations, which then further promotes the need for therapy with another antibiotic.

Infectious diseases researchers in pursuit of new antibiotics have employed several strategies in attempts to overcome the numerous resistance mechanisms bacteria have come to evolve. These have included the modification of existing drug entities, as seen with clarithromycin, azithromycin, and telithromycin (derived from erythromycin A) or tigecycline (derived from minocycline) or the use of various combination therapies, such as trimethoprim-sulfamethoxazole, or amoxicillin in the presence of the β-lactamase inhibitor clavulanic acid.

Of direct pertinence to the work we are to describe in this Award Address, the discovery of truly novel antibacterial classes remains a goal of utmost interest to those working in infectious diseases research. This is because it is with such agents, particularly those having a new mechanism of action (MOA), that one may best hope to avoid the problems of shared cross-resistance between prior therapeutic agents, as is highly common for members of the same family of antibiotics.

The discovery of the oxazolidinone linezolid (**1**, PNU-100766, formerly U-100766, Figure 1) is the subject of this Team Innovation Award address.[7] The Food and Drug Administration (FDA) approval of linezolid in April 2000 represents the advent of the first completely new class of antibiotics to have been developed in the over 35 years since the progenitor quinolone antibiotic, nalidixic acid, was approved in 1963. In 2003, the cyclic lipopeptide daptomycin gained regulatory agency approval and thus represents, to date, the only other subsequent new class of antibiotics to reach the market.[8]

The work to be described in the program that led to the discovery of linezolid occurred at the Upjohn Company, a legacy company of Pfizer Inc.[9,10] This was initiated soon after one of us heard the first public presentation made by scientists at E. I. du Pont de Nemours & Company on their discovery of the oxazolidinone antibacterial agents. That disclosure took place at the 1987 meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy in New York City, where Dr. A. M. Slee and co-workers reported on two intriguing 5-(*S*)-acetamidomethyl-2-oxazolidinone leads, identified as DuP-105 (**2**) and DuP-721 (**3**, Figure 2).[11] These compounds had several attractive characteristics, including (1) potent in vitro activity and oral efficacy in animals against multidrug resistant (MDR) Gram-positive bacteria, such as MRSA and *Mycobacterium tuberculosis*. (2) DuPont researchers were reportedly unable to induce bacteria to become resistant to these compounds in vitro. (3) The compounds demonstrated encouraging pharmacokinetic (PK) properties in rodents, being both parenterally and orally active. (4) They comprised a novel class of totally synthetic antibacterial agents, operating by a unique MOA involved with the inhibition of bacterial protein synthesis.

The early DuPont structure–activity-relationships (SAR) had defined a number of significant features of the oxazolidinone pharmacophore: (1) the importance of the *N*-aryl group, (2) the optimal activity of a C-5 acetamidomethyl group, (3) the requirement of the *S*-configuration at this C-5 position for antibacterial activity, (4) the finding that electron-withdrawing groups in the aryl para position often provided optimal activity, and (5) additional substitutions at the aryl ortho position or C-4 of the oxazolidinone ring usually had a detrimental, or at best, indifferent effect on the antibacterial activity.[12]

Immediately following the initial disclosure of the two DuPont oxazolidinones at ICAAC in late 1987, we in the Brickner laboratory at the Upjohn Company began to explore opportunities to find new proprietary series of oxazolidinones. Thus began a small part-time lead-finding project with a total of three chemists, taking full advantage of a company policy that provided scientists the individual opportunity to explore ideas in areas of research tangential to, or completely disconnected from, their primary project.

Within a period of about 18 months, our own SAR studies had successfully advanced to the point where we had discovered several new oxazolidinone subclasses. These oxazolidinones originated from considerations of enhancing binding interactions by restricting rotational degrees of freedom (1) for the aryl methyl ketone of **3** by its incorporation into five- or six-membered benzo-fused cyclic ketones (e.g., **4**) or (2) about the bond connecting the oxazolidinone nitrogen and the aryl ring by constructing rigid [6,5,5]-tricyclic-fused oxazolidinones (e.g., **5,** Figure 3).[9] A third theme was to dispense with the cyclic ketone by utilizing a surrogate amide incorporated on a 5′-indoline ring (e.g., **6**).[13]

While indeed certain [6,5,5]-tricyclic oxazolidinones demonstrated intriguing antibacterial activity, such as pyridyl derivative **5**, which demonstrated efficacy equivalent to that of vancomycin in an in vivo model of a *S. aureus* infection,[9] here we shall focus on only the cyclic ketones and 5′-indolinyl oxazolidinones, as it was from findings with these series that we began to define a path forward from the earliest stages of our project. What follows is a perspective on how our team proceeded from these early leads toward the ultimate discovery

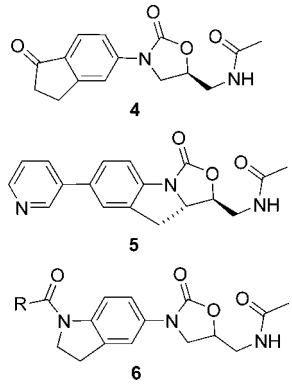


Figure 3. Key early Upjohn lead oxazolidinones.

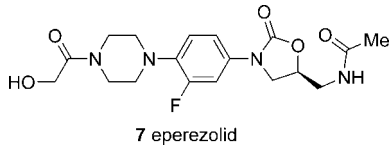


Figure 4. Structure of clinical candidate eperezolid.

of Upjohn's two clinical candidates, **1** and **7** (eperezolid, PNU-100592, formerly U-100592, Figure 4).[14]

From the investigation of the aforementioned study of benzo-fused alicyclic ketones we became most interested in the indanone oxazolidinone (±)-**4**, and it would prove to be a pivotal compound for this program. This cyclic version of the most active DuPont lead demonstrated in vitro antibacterial activity and oral in vivo efficacy in a murine model of MRSA infection that were commensurate with those of (±)-**3**, as well as similar PK properties in the rat. Not long after our in vivo pharmacology colleagues had identified these encouraging attributes of **4**, we became aware of some disquieting news. In early 1989, we heard fragmentary reports that the DuPont group had exited the oxazolidinone area, apparently because of some toxicity encountered in their preclinical animal studies.[15] This information was a disconcerting blow to our nascent program and mandated that we find a means to rapidly assess whether there were prospects for continuing on with the oxazolidinones. It also placed a high emphasis on the need for us to demonstrate a differentiated safety profile for one of our promising compounds then in hand, relative to **3**. In light of the urgency for gaining an understanding around this safety question, we were extremely fortunate to initiate a collaboration with one of our colleagues, Dr. Richard Piper, a pathologist who provided great leadership in seeking to resolve this matter and who volunteered to run studies in his laboratory (again taking advantage of Upjohn's 10% free-time policy). There were two desired outcomes for those studies: (1) establish the existence of a structure−toxicity relationship (STR) within the oxazolidinone series and (2) identify a viable means of focusing, as earliest as possible, on those oxazolidinone subclasses having the most favorable safety profiles.

Piper designed a highly practical toxicological protocol that would prove to serve us very well in reaching those goals. He opted for a comparative study of month-long duration in the rat, which normally would have necessitated the chemists to prepare large quantities of the test substances. However, as his protocol was both compound- and animal-sparing, we needed only to synthesize approximately 10 g each of the comparator (±)-**3** and (±)-**4**, our compound with the best overall profile at that time. The test substances were administered daily to three animals per sex in parallel evaluations, dosed orally at 100 mg/kg b.i.d., a level that represented a 10-fold increase above the ED_{50}, the amount of drug found to protect 50% of the mice in a *S. aureus* lethal systemic infection model. Throughout most of the study, those animals dosed with (±)-**3** did not fair well; there was observed severe progressive weight loss and one death, with an additional two animals in a moribund state being euthanized. Upon necropsy, bone marrow toxicity was detected, along with other negative findings. In contrast, there were only few, mild adverse effects observed in the animals treated with (±)-**4**. There were no negative clinical signs, and the serum and urine chemistries were normal. Nor was there any histopathological evidence of drug-related toxicity.[16] This favorable outcome for our lead compound represented a striking differential in the toxicological profiles of two similar oxazolidinone aryl ketones, one acyclic and the other integrated into an appended ring.

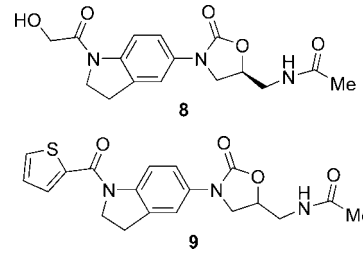


Figure 5. Structures of 5′-indolinyloxazolidinones.

While we could not have been more satisfied with this outcome, we would ultimately learn that the very structure corresponding to **4** had subsequently appeared in a U.S. patent awarded to DuPont. While an obvious disappointment, they had filed their patent application prior to our own. Nonetheless, we were able to take considerable consolation from the knowledge that this indanone had demonstrated the existence of an oxazolidinone STR. Had we not had **4** in hand at that critical time, there was a significant probability the program could have been terminated had the outcome with another derivative been less favorable. This admission of serendipity notwithstanding, the contrasting findings with (±)-**4** vs (±)-**3**, two compounds differing by seemingly subtle structural characteristics, validated the usefulness of the multiday protocol as a strategy we could use, and would come to rely on, in our resulting search for other well-tolerated oxazolidinones.

One such compound emerged shortly thereafter from our investigation of a series of 5′-indolinyl oxazolidinones. Compound (±)-**8** (PNU-85112,[13] Figure 5) displayed in vitro and in vivo antibacterial activity that was similar to that of the comparator (±)-**3**. This derivative was very well tolerated when dosed orally at 100 mg/kg b.i.d. for 30 days in the rat and thus became recognized as the first oxazolidinone to demonstrate that compounds with a nitrogen atom in the para position on the phenyl ring could have an excellent safety profile. This concept was subsequently validated when we conducted safety studies on its single active 5-(*S*) enantiomer **8** (PNU-97456[13]), as well as **9** (Figure 5), a 5′-indoline bearing an *N*-acylthienyl moiety, as both performed very well in the 30-day toxicological studies.

These very early preclinical month-long animal toxicity studies established STRs that proved critical to the continuation of the Upjohn oxazolidinone project and, ultimately, our success in finding the two clinical candidates, eperezolid and linezolid. While this is now more common, at the time this was an innovative approach to drug discovery. With the success of linezolid, the approach represents, to our knowledge, an

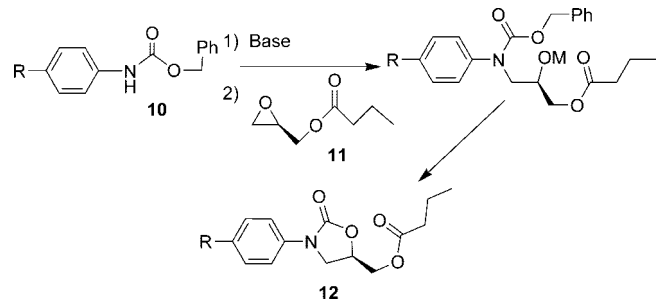
unprecedented example of driving a discovery program to deliver a first-in-class drug to the market, using multiple, early preclinical animal safety evaluations.

Following these several successful demonstrations that the safety protocol was viable for advancing the oxazolidinone project, the chemistry team that had been initially made up of the Brickner laboratory was expanded in 1990 to also comprise the Hutchinson laboratory, and then in early 1991 the Barbachyn laboratory, at the time the oxazolidinone project was formally approved by senior discovery management. Along with our biology colleagues in the laboratories of Gary Zurenko and Dr. Charles Ford, and also our clinician Donald Batts, M.D., the team formulated a set of distinct goals and a strategy for identifying compounds having vancomycin-like activity and other attributes worthy of entering clinical trials. Given that the oxazolidinones represented a new class of antibacterial agents with an unknown human toxicological profile, we sought ideally to identify more than one drug candidate for phase I studies to maximize our chances for success in advancing a compound to a new drug application (NDA). Our laboratory objectives were focused on finding compounds with in vitro and in vivo activity vs MRSA that was comparable to or better than vancomycin, the agent of last resort. Given the severe nature of many invasive nosocomial Gram-positive infections, it was deemed absolutely critical that the drug have both intravenous (iv) and oral formulations. This would require finding compounds having physicochemical properties that could provide the sufficiently high aqueous solubility needed for the iv formulation and yet maintain an acceptable degree of oral bioavailability, good human PK allowing either q.d. or b.i.d. dosing regimens, and (of course) an acceptable safety profile.

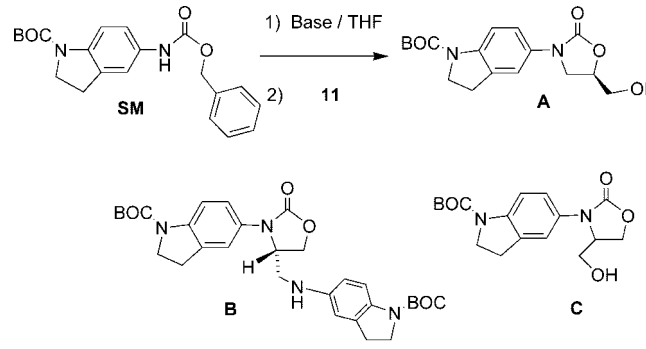
From the chemistry team's perspective, another critical objective was to find a practical and efficient synthetic route to the enantiomerically enriched 5-(*S*)-acetamidomethyloxazolidinones. All of our initial synthetic workup to that point had utilized racemic compounds, a strategic decision we had made to accelerate the SAR advancement and identification of proprietary matter in the initial small team. These racemic aryloxazolidinones were constructed from the corresponding *N*-allyl-*N*-carbobenzyloxy (CBZ) aniline by means of an iodocyclocarbamation reaction.[9a] The DuPont synthesis of optically active **3** employed an oxazolidinone cyclization method first described in the racemic form by Herweh and Kauffman.[17] The DuPont version entailed the high-temperature reaction of an aryl isocyanate with the commercially available chiral epoxide (*R*)-glycidyl butyrate (**11**) to give the butyrate ester of the 5-(*R*)-hydroxymethyl oxazolidinone.[18] Following saponification, the alcohol was carried on in several steps to **3**. For our program, use of this approach would have necessitated accessing a number of commercially unavailable substituted aryl or heteroaryl isocyanates; typically these would be prepared by treating the corresponding aniline with phosgene. This method presents two problems: the first involves the inherent safety hazards in using phosgene on a sizable scale, particularly in a discovery research laboratory environment; the second is incomplete conversion due to formation of an equivalent of the hydrochloride salt of the aniline.

As depicted in Scheme 1, our proposed alternative route involved functionalizing the appropriate aniline with a CBZ group, deprotonating the carbamate (**10**) N−H with a suitable base, and then alkylating with **11**. Further, it was envisioned that the resulting alkoxide anion would cyclize onto the carbamate carbonyl with expulsion of benzylalkoxide to yield the desired oxazolidinone **12**, with a butyrate side chain on the

Scheme 1. Synthetic Route to Chiral Oxazolidinones



Scheme 2. Reaction Profile with Various Bases



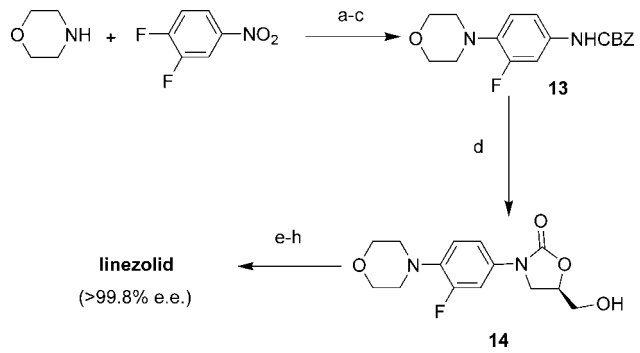
Base	A	B	C	SM
NaH	27%	15%	9%	22%
KH	8%	53%	12%	20%
n-BuLi	90%	0%	0%	0%

5-methyl substituent. In the first attempt to put this to practice, upon use of NaH as the base the course of the reaction was highly discouraging, with a plethora of spots generated on the TLC plate. As a consequence, efforts were begun to consider other routes that might prove to be more practical.

At this juncture in our account of some of the innovations encountered in the linezolid discovery story, some commentary is in order concerning an important individual contribution from Peter Manninen, which as a direct consequence of his innovation and its significance, resulted in his sharing this Team Innovation Award with the co-inventors of linezolid. This entailed a personal initiative where he also independently conceived the oxazolidinone forming reaction, and even though he was informed that the single attempt with sodium hydride had failed, he evaluated the reaction further, leading to his discovery of a highly successful reaction. By substituting *n*-BuLi for the NaH base, Manninen found the reaction proceeded at low temperature in very high yield and exactly as originally conceived—other than the compound he isolated in high optical purity was the 5-(*R*)-hydroxymethyloxazolidinone (**A**, Scheme 2), the result of in situ cleavage of the butyrate ester **12**. In the most optimal case found as we subsequently explored the scope of the reaction, using *N*-phenylcarbamic acid methyl ester as the substrate on 0.16 mol scale, 3-phenyl-5-(*R*)-hydroxymethyl-2-oxazolidinone was readily obtained in 95% yield and 98% enantiomeric excess (ee).[19] This general approach soon came to be referred to in our team as the "Manninen reaction", where it proved to be of quite broad utility for the synthesis of many varied oxazolidinone templates.

There are several factors that appear important in directing this lithium-mediated reaction down the desired pathway. Following the cyclization to the oxazolidinone, the equivalent

Scheme 3. Initial Synthetic Route for Linezolid[a]



[a] Reagents and conditions: (a) $(i\text{-Pr})_2$NEt, EtOAC, reflux, 99.6%; (b) HCO_2NH_4, Pd/C, THF/MeOH, 96%; (c) CBZCl, $NaHCO_3$, aqueous acetone, 98.6%; (d) *n*-BuLi, THF, −78 °C, epoxide **11**, 81.4%; (e) MsCl, Et_3N, CH_2Cl_2; (f) NaN_3, DMF; (g) Pd/C, H_2, EtOAc; (h) Ac_2O, pyr, 68.8% from **14**.

of lithium benzylalkoxide that is generated serves to saponify the butyrate ester (e.g., **12**), giving the 5-lithioalkoxymethyl-2-oxazolidinone and benzyl butyrate. This in situ transesterification is a potentially reversible reaction, but we believe the equilibrium established between the two butyrate ester species is driven far in the desired direction by precipitation of the lithium salt of the 5-(*R*)-hydroxymethyl-2-oxazolidinone.

Other factors affecting success of the *n*-BuLi-mediated reaction were gleaned by investigating various aspects of the original failed cyclization attempt. It was established that with NaH or $NaN(SiMe_3)_2$ at THF reflux, some of the desired 5-(*R*)-hydroxymethyloxazolidinone (as illustrated with the substituted indoline **A**) was formed, but a significant isolated byproduct was a quite unusual 4-arylaminomethyl-2-oxazolidinone **B**, and to a lesser extent, the 4-hydroxymethyl oxazolidinone **C**, apparently resulting from nonselective epoxide ring opening (Scheme 2). Most interesting was that in using either KH or $KN(SiMe_3)_2$, **B** became the predominant product isolated.[20]

In additional studies that we will not detail here, we demonstrated the critical role played by the Li^+ cation in the successful cyclization. In contrast to the elevated temperatures required to observe complete conversion with the Na^+ or K^+ counterion bases, the presence of the Li^+ cation appears to both promote the low-temperature epoxide-opening by the nucleophilic carbamate nitrogen and to direct the highly regioselective nucleophilic attack on the epoxide. This we attribute to the ability of the lithiated carbamate's cation to function as a Lewis acid through multidentate coordination with the epoxide's oxygen and an oxygen center on the butyrate ester. By addition of LiBr to the reaction with $NaN(SiMe_3)_2$ conducted at THF reflux, the desired cyclization was realized, albeit in a more modest yield than with *n*-BuLi. No **B** was detected, in contrast to the case when the reaction is conducted in the absence of LiBr.

To illustrate the utility of the Manninen cyclization, **13** on a small scale gave **14**, the requisite intermediate to linezolid, in 81.4% yield within our discovery laboratory. Overall, the eight-step synthesis provided linezolid in 55% overall yield and >99.8% ee, beginning with the addition of morpholine to 3,4-difluoronitrobenzene (Scheme 3).[19] This same cyclization process was amenable to pilot plant scale synthesis and was utilized on a 100 kg scale with only minimal modification to the overall route to produce the first clinical lots of eperezolid and linezolid. In summary, the Manninen reaction proved to be highly advantageous in (1) obviating the need for isocyanate

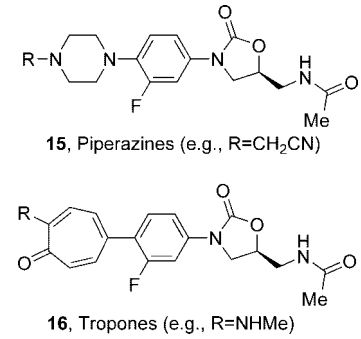


Figure 6. Structures of representative piperazinyl- and troponyloxazolidinones.

synthesis and thereby avoiding the objectionable use of phosgene, (2) avoiding the need for high temperature cyclization conditions found in the Herweh−Kauffman reaction, and (3) being general, mild, efficient, and readily scaleable.

That said, eventually an improved process was developed in concert with Pharmacia & Upjohn's Process Chemistry group,[21,22] which involved using LiO-*t*-Bu to deprotonate **13** and alkylating with (*S*)-3-chloro-1,2-propanediol in place of **11**. Another significant improvement they developed was in the conversion of the 5-(*R*)-hydroxymethyl group to the requisite 5-(*S*)-acetamidomethyl side chain, involving the treatment of the intermediate 3-nitrobenzenesulfonate with a large excess of NH_4OH followed by acetic anhydride. This improved seven-step process gave linezolid in 65% overall yield.

As our three chemistry laboratories became fully deployed on the oxazolidinone effort, the expanded team was successful in identifying a total of approximately 25 proprietary oxazolidinone series of interest. However, by the end of 1992, three of those series had risen to the forefront based on their individual compelling attributes. These were the piperazinylphenyloxazolidinones (**15**, emanating from the Hutchinson laboratory),[23] the 5′-indolinyloxazolidinones (**6**, Brickner laboratory),[9a,13] and the troponylphenyloxazolidinones (**16**, Barbachyn laboratory,[24] Figure 6). Barbachyn demonstrated that a distinct potency advantage could be recognized by fluorinating the phenyl group of these troponyloxazolidinones, and this significant finding would hold for a number of other series examined, including the templates yielding our two drug candidates. While very potent, these troponylphenyl analogues unfortunately had poor aqueous solubility and were the most difficult of the three series to synthesize. Furthermore, some tropone analogues displayed poor PK parameters in rodents, and some derivatives demonstrated signs of toxicity.[25] Whereas the 5′-indolinyloxazolidinones generally possessed a superior safety profile, on the whole, the level of in vitro potency and in vivo efficacy seen with the lead **8** was not quite as robust as was ultimately desired in our targeted product profile.

In contrast, the overall profile of the piperazinylphenyloxazolidinone series appeared considerably more favorable. The synthesis of this series was the most facile of the three, and the distal piperazine nitrogen provided a convenient synthetic handle allowing considerable flexibility in analogue generation. As previously noted, the requirement to have both iv and oral formulations was a paramount goal, and many of the piperazinylphenyl analogues demonstrated very good aqueous solubility. Importantly, selected analogues exhibited a good safety profile in the 30-day toxicological evaluation in the rat.

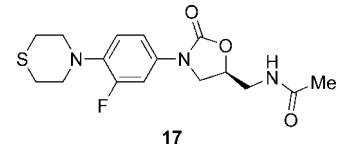
The origin of Hutchinson's idea to incorporate a piperazine ring congealed from considerations that the piperazine moiety could sometimes improve solubility in other pharmacophores

and the aforementioned SAR finding from the 5′-indolines that certain compounds with nitrogen positioned in the para phenyl position were well tolerated. Additionally, there was the awareness that the piperazine ring could serve as a bioisosteric replacement for a pyridyl group in the fluoroquinolone antibacterial agents (e.g., ciprofloxacin), and DuPont had prepared a 4-(4-pyridyl)phenyl-5-(*S*)-acetamidomethyl-2-oxazolidinone (E-3709) that was reported to have superior activity to **3**.[26]

On the basis of the above-mentioned features, our team collectively resolved to focus all of our medicinal chemistry efforts exclusively on the piperazinylphenyl subclass, with a goal to rapidly optimize the SAR to identify one or more viable drug candidates. This particular type of close collaborative action was an innovative step at Upjohn. Our three laboratories demonstrated a very high level of teamwork that involved not only a great deal of exchange of chemical intermediates, plans, and know-how but in particular ideas for new targets. We frequently held group brainstorming sessions, and individual chemistry team members would volunteer to synthesize specific prioritized compounds with little consideration being given regarding where the idea had originated.

Because a description of the full SAR[23] of the piperazinylphenyloxazolidinones is clearly beyond the scope of this discussion, we focus here directly on a particular derivative that led to eperezolid, a compound having an acetoxyacetyl moiety appended to the piperazine distal nitrogen. Of the many substituted piperazines examined, this was a highly intriguing analogue that displayed excellent in vivo oral antibacterial efficacy at a level exceeding what was anticipated from its in vitro minimum inhibitory concentration (MIC). Given the observed in vivo result, we hypothesized that in the mouse the compound's acetate underwent cleavage by one or more mammalian esterases to release the more active component, presumably the hydroxyacetyl derivative. As previously noted, this is the very same substituent found most favorable in the indoline series. As anticipated, when we prepared and tested **7**, it indeed was found to have excellent in vitro potency[27] and excellent oral in vivo efficacy versus *S. aureus* and *S. pneumoniae* in murine infection models, on par with vancomycin dosed subcutaneously.[28] Eperezolid underwent extensive profiling, and on the basis of its overall outstanding properties, including high solubility (4.2 mg/mL) in pH 7 phosphate buffer, it emerged as the most interesting piperazine analogue and was selected as the first Upjohn oxazolidinone clinical candidate. Phase I trials with eperezolid commenced in October 1994 in Kalamazoo, MI, and were successfully completed, with the drug being well-tolerated when dosed up to 2000 mg given orally q.i.d. for 14.25 days. The most common clinical observations noted were gastrointestinal symptoms and yeast superinfections.[7d,29]

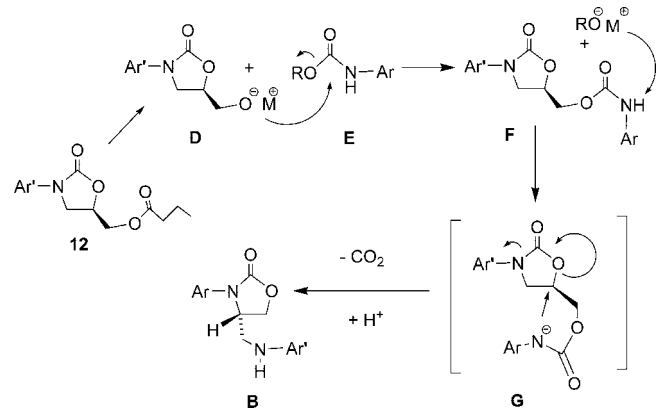
Eperezolid had approximately 2-fold enhanced in vitro activity against the enterococcal and streptococcal pathogens tested compared to the corresponding des-fluoro analogue. Addition of a second fluorine to the 5-phenyl position of eperezolid resulted in a further 2-fold enhancement in the in vitro potency in *S. aureus* and *S. pneumoniae* and retained the excellent in vivo antibacterial efficacy. However, this derivative was accompanied by a nearly 40% reduction of the aqueous solubility relative to eperezolid, and given the importance of high aqueous solubility, the reduced solubility was only marginally acceptable for the iv formulation. Additionally, the synthesis of the 3,5-difluorophenyloxazolidinone template was more complicated than the monofluorophenyl template, and these combined factors drove the decision to move forward only with the 3-fluorophenyloxazolidinone series.



17

Figure 7. Structure of thiomorpholineoxazolidinone.

Scheme 4. Proposed Mechanism of Byproduct **B** Formation



Because of the interest in examining the morpholine bioisosteric replacement for the piperazine ring system, championed in particular by Barbachyn, we prepared linezolid.[9,14,15] This work was carried out in parallel temporally with the piperazinylphenyl subclass SAR optimization; in fact, eperezolid and linezolid were synthesized for the first time within only a 2-day period in early 1993.[7a] Linezolid also met our key requirement of good solubility, which was found to be 3.7 mg/mL in pH 7 phosphate buffer. Following extensive profiling, linezolid demonstrated in vitro antibacterial potency and an efficacy profile that was quite similar to that of eperezolid, although with some strains linezolid was 2-fold less potent than eperezolid.[27,30] Like eperezolid, linezolid compared very well with vancomycin in the mouse models of infection of *S. aureus* and *S. pneumoniae* and demonstrated good oral and subcutaneous activity vs VRE infections.[28] Resistance to linezolid was shown to develop slowly in vitro via multistep mutations, with a frequency of resistance in *S. aureus* ATCC 29213 being $<8 \times 10^{-11}$.[30] Both clinical candidate compounds were found to be bacteriostatic against the enterococci and staphylococci but bactericidal against most strains of streptococci.

Another interesting compound closely related to linezolid was also prepared during this same time period. While not strictly a bioisostere, the thiomorpholine congener **17** (PNU-100480, formerly U-100480, Figure 7) was found to have potent activity against *M. tuberculosis* (e.g., strain H37Rv MIC $\leq$ 0.125 μg/mL vs isoniazid 0.2 μg/mL), including activity against MDR strains.[31,32] Although **17** demonstrated very good activity against the Gram-positive pathogens of interest, its nearly 20-fold lower aqueous solubility (0.2 mg/mL) than linezolid, as well as a more complex metabolic profile observed in rodents, eliminated this more lipophilic derivative from further consideration as a drug candidate for the Gram-positive indications.

At the time we began the oxazolidinone project, the detailed aspects of the oxazolidinone MOA were not well understood. Throughout the intervening 20 years, a number of research groups have published various sets of data that have led to sometimes conflicting conclusions concerning the binding site or mode of action of the oxazolidinones.[7g,33–40] It is helpful to set the stage for discussing the current understanding of the oxazolidinone MOA with a brief description of the key aspects

of the early steps involved in prokaryotic ribosomal protein synthesis. The intact bacterial 70S ribosomal unit is an impressive and huge multicomponent "machine" that can manufacture proteins at the rate of 15 amino acids a second and is assembled from mRNA and two subunits, the small (30S) and large (50S) subunits. Together these subunits are made up of a total of three ribosomal RNA (rRNA) strands and over 50 proteins. In what is admittedly an oversimplified description omitting the role of other critical components such as GTP and elongation factors, etc., the process of translation begins with the binding of the 30S subunit to mRNA, which then assembles together with the initiator fMet-tRNA and the 50S subunit to form the functional 70S initiation complex. Peptidyl bond formation in the elongation cycle is catalyzed by rRNA at the peptidyl transferase center (PTC). There, an appropriate aminoacyl-tRNA (aa-tRNA), guided by proper matching of the mRNA codons with the aa-tRNA anticodon, docks into the A site, where it couples to the growing peptide chain in the P site. Translocation then shifts the peptidyl-tRNA to the P site, and the empty or deacylated-tRNA shifts to the E site.[41]

From in vitro cell-free studies, it has been concluded that the oxazolidinones function to inhibit the formation of the 70S initiation complex.[28] A recent publication by former Pharmacia colleagues in collaboration with Professor Alexander Mankin at the University of Illinois at Chicago[42] has presented data generated from the interaction of three oxazolidinones within living *S. aureus* cells. These studies provide strong evidence supporting a more detailed understanding of the target binding site of the oxazolidinones, derived from three separate cross-linking experiments, each using a different biologically active, radiolabeled oxazolidinone photoaffinity probe to triangulate the orientation of the oxazolidinone on the ribosome. These data point to the oxazolidinones binding at the A site within the PTC, and thus, they interfere with docking of the aa-tRNA. As a result, bacterial protein synthesis is shut down at a very early stage, in a distinctive manner such that linezolid is not cross-resistant with any other currently marketed antibiotic. Recently, in work conducted at Pfizer, it has been found that some of the few known clinical isolates of linezolid-resistant *S. aureus* are cross-resistant with several investigational "truncated" Hygromycin A analogues, semisynthetic compounds that have demonstrated potent in vitro and in vivo Gram-positive antibacterial activity.[43] This finding implies that they likely have a similar MOA as the oxazolidinones.

In the period when eperezolid and linezolid were undergoing extensive preclinical evaluations, we were uncertain as to which preclinical species, rat or dog, would best predict the human PK. When dosed orally in the rat at 25 mg/kg, linezolid clearly displayed a superior PK profile over eperezolid, demonstrating excellent oral bioavailability (109% vs 56%, respectively). In the dog, however, both compounds performed very well, with oral bioavailabilities of 100% (eperezolid) and 97% (linezolid).

In the 30-day animal safety studies, the no-observed-adverse-effect-level (NOAEL) in the rat and dog species was 25 (mg/kg)/day for eperezolid[44] and 20 (mg/kg)/day for linezolid.[45] For linezolid, oral doses of 50 (mg/kg)/day in the rat and 40 (mg/kg)/day in the dog were well tolerated over a 30-day evaluation period, with only mild adverse effects observed. In similar toxicological evaluations conducted with eperezolid, only minimal adverse effects were observed in both species at 80 (mg/kg)/day.

Linezolid followed eperezolid into phase I clinical trials in April 1995, just 6 months later, in studies conducted in the U.K. Fasted healthy adults administered a single 375 mg dose of linezolid by iv or po were found to have comparable exposures to drug in the blood and nearly superimposable areas under the concentration time curves over the course of the monitored 24 h period. Additional PK studies in healthy volunteers dosed orally with 625 mg linezolid b.i.d. for 14 days established that the steady-state plasma concentrations exceeded the MIC_{90} values for staphylococci, enterococci, and pneumococci for the entire 12 h period following the final dose. Mean peak concentrations in the plasma reached about 17 μg/mL, and mean trough concentrations were about 7 μg/mL; the latter value exceeds the MIC_{90} for *S. aureus* strains by nearly 2-fold. These PK findings were far superior to those for eperezolid; e.g., the mean plasma concentration of eperezolid (estimated to be the peak concentration) collected 2 h after the last dose of 2000 mg, given q.i.d. to healthy volunteers, was 9 μg/mL.[46]

The oral bioavailability of linezolid in humans is 100%, and the drug is rapidly and completely absorbed. While a slight decrease in the absorption rate was noted when linezolid was dosed with food, there was no diminution of the extent of absorption. The volume of distribution of linezolid in humans is approximately that of total body water (40–50 L), and the elimination half-life is 5–7 h. Linezolid has a high free fraction, with only 31% bound to human plasma proteins. The total clearance of linezolid in humans is low (100–200 mL/min), with a renal clearance of 30–50 mL/min and a nonrenal clearance of 70–150 mL/min. The percentage of dose found in the urine as the parent drug is 20–30%.[47]

The metabolic profile of linezolid is particularly interesting, with three metabolites formed in humans. Two of those metabolites result from oxidative cleavage of the morpholine ring. These oxidations are not mediated by cytochrome P450 (CYP_{450}) enzymes and are hypothesized to involve nonenzymatic oxidation by reactive oxygen species such as superoxide.[47] Linezolid was not found to be an inhibitor or inducer of CYP_{450} isozymes.[48]

Other favorable PK features of linezolid are those relating to tissue penetration. In a study examining the concentration of linezolid in human lung epithelial lining fluid (ELF) following a total of five oral doses of 600 mg b.i.d. in healthy volunteers, the mean ELF peak concentrations measured 4 h after the last dose reached 64.3 ± 33.1 μg/mL, significantly exceeding mean peak plasma concentration of 7.3 ± 4.9 μg/mL.[49] In a rat study with ^{14}C-labeled linezolid dosed either po or iv, the radiolabeled drug was found to be widely distributed, with levels in most soft tissues similar to drug concentrations in the blood.[50]

On the basis of its superior human PK profile relative to eperezolid (vide supra), linezolid was selected for advancement into clinical trials in infected patients. In phase III trials, linezolid proved to have excellent efficacy in the following indications (observed clinical efficacy for linezolid vs comparator drug): outpatient community-acquired pneumonia (CAP) (90% vs 91%, cefpodoxime),[51] hospitalized CAP (91% vs 89%, cetriaxone/cefpodoxime), hospital-acquired pneumonia (66% vs 68%, vancomycin),[52] uncomplicated skin and soft tissue infections (SSTI) (91% vs 93%, clarithromycin), complicated SSTI (89% vs 86%, oxacillin/dicloxacillin),[53] MRSA infections (77% vs 74%; vancomycin), and VRE infections (88%).[15,54]

It is noted that in the clinical trial for the latter indication, no comparator drug is listed, as none existed as an approved therapy for treatment of VRE infections. Therefore, Pharmacia & Upjohn researchers worked in collaboration with FDA regulatory personnel to design a suitable dose-ranging clinical trial protocol in which a differential treatment outcome was expected between patients treated with 200 mg vs 600 mg b.i.d. of

linezolid.[55] Given the successful outcomes from all of the above studies, Pharmacia proceeded to file an NDA, and linezolid was approved by the FDA on April 18, 2000. Following the date of first synthesis in early 1993, linezolid entered phase I trials almost exactly 2 years later. The phase II studies were initiated in mid-1996, and more extensive phase III trials began in January of 1998. Overall, there was a 54-month span from first-in-human studies to the filing of the NDA.

Linezolid has been approved in the U.S. for the treatment of the following indications: for nosocomial and community-acquired pneumonia caused by *S. aureus* (methicillin-susceptible or MRSA) or *S. pneumoniae* (penicillin-susceptible or multi-drug-resistant strains) and vancomycin-resistant *E. faecium* (including concurrent bacteremias). Linezolid has been approved for use in children and newborns against Gram-positive infections. Linezolid is also approved for treatment of complicated skin and skin-structure infections including those due to MRSA. These include Gram-positive bacterial diabetic foot infections (MRSA) without concomitant osteomyelitis. Linezolid became the only approved agent for treatment of hospital-acquired MDR *S. pneumoniae* infections and is the first and only oral drug approved for the treatment of VRE infections. It is available as interchangeable formulations of 600 mg in iv bag, tablet, and oral suspension.

Linezolid is generally considered to be well tolerated in humans,[56] and the most common side effects observed in the clinical trials were (percent incidence) diarrhea (2.8–11%), nausea (3.4–9.6%), and headache (0.5–11.3%).[55] With longer-term usage of linezolid, there is an association of reversible myelosuppression (anemia, thrombocytopenia, leukopenia, or pancytopenia), particularly when the course of therapy exceeds 2 weeks.[55] As a result, weekly monitoring of total blood count status is recommended. Lactic acidosis has been reported in some patients treated with linezolid,[57,58] and there have been reports of peripheral and optic neuropathies,[59,60] primarily with patients treated with linezolid beyond the maximum recommended duration of 28 days, but the causal relationship with linezolid treatment has not been established.[61] Linezolid is a weak, reversible, and nonselective inhibitor of monoamine oxidase-A ($K_i = 55\ \mu M$)[62] and has the potential for interacting with serotonergic and adrenergic agents. There are reports of serotonin syndrome in patients coadministered linezolid with a selective serotonin-reuptake inhibitor antidepressant.[63] Patients administered linezolid have been advised to avoid intake of large quantities of food or beverages having a high tyramine level, such as aged cheese, beer, or red wine.[55]

In summary, linezolid is the first clinically useful oxazolidinone antibacterial agent to be approved for use in the treatment of infections caused by sensitive and multidrug-resistant Gram-positive pathogens. It inhibits bacterial protein synthesis with a mechanism that appears to have unique aspects such that it lacks cross-resistance with all other marketed antibiotics. The availability of both oral and parenteral formulations provides the potential for shorter duration iv therapy than vancomycin, by switching to the oral formulation without need for dosage adjustments. Linezolid is generally well tolerated and has, to date, been used in an estimated 3 million patients.

Acknowledgment. The authors are deeply honored by the receipt of the 2007 Team Innovation Award and are most privileged and fortunate to have our work so recognized by the American Chemical Society. The successful discovery and development of linezolid were only made possible by the dedication, perseverance, and insights of the many talented multidisciplinary scientists with whom we have worked and have been fortunate to consider as both friends and colleagues. It is with immense appreciation of them that we also view this Award as an acknowledgement of so many other colleagues, whose diligent efforts have also significantly contributed to the discovery and development of linezolid. It is our hope that each one of them also shares in the sense of deep personal reward that comes with the knowledge that our collective accomplishments have resulted in a useful antibiotic that has helped thousands of patients. As with any attempt to recount historical aspects of such an extensive undertaking, there is an inherent risk of omitting from proper attribution individuals rightfully deserving of mention. While we have sought to identify those key research personnel whose work significantly impacted the discovery of linezolid, an apology is extended in advance to any individual who has inadvertently been omitted. Richard Piper, John Palmer, and Thomas Platte were absolutely critical to the success of the Upjohn oxazolidinone program in designing and conducting the early toxicology work, which proved to be so essential to the success of this research program. We are indebted to our other chemistry team colleagues Debra Allwine, Kristine Garmon, Stuart Garmon, Kevin Grega, Susan Jacobsen, Toni Poel, Ray Reid, and Dana Toops and to David Houser, who prepared the multi-kilogram bulk drug lots of the two drug candidates. Equally critical to the success of this program were our biology colleagues who conducted innumerable in vitro MIC determinations: Gary Zurenko, working with Ronda Schaadt, Betty Yagi, and John Allison and the in vivo pharmacology lab of Charles Ford, who worked with Judith Hamel, Judy Moerman, and Douglas Stapert. MOA studies were conducted by Jerry Buysee, Keith Marotti, Dean Shinabarger, and William Demyan. Another highly critical aspect of any drug discovery process is involved with assessing the PK properties, and John Greenfield, Neil Duncan, Ian Martin, Martin Howard, Peter Daley-Yates, and Dennis Stalker were instrumental in preclinical and clinical PK determinations. Finally, we gratefully acknowledge the clinical development work carried out by Dr. Donald Batts and Dr. Steve Pawsey, and we gratefully acknowledge Susan Speziale, who guided us as clinical development project manager.

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