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Process Development and Preparation of 2-Deoxyparaherquamide: Implementation of a Selective Reduction of Secondary Amides on a Kilogram Scale

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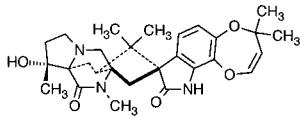
Abstract:

The preparation of 2-deoxyparaherquamide, PNU-141962, is accomplished through the indirect selective reduction of a secondary amide in the presence of a tertiary amide. The kilogram-scale process is safe and robust and allows for the isolation of analytically pure material in high overall yield without a chromatographic purification.

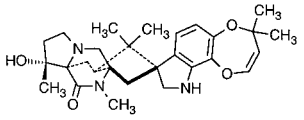
Introduction

The anthelmintic drug candidate, 2-deoxyparaherquamide,[1] PNU-141962 (**5**), is derived from the fermentation product paraherquamide,[1] PNU-97333 (**1**). Although these compounds differ only in oxygenation at C-2, the much superior toxicity profile of PNU-141962 made it a more attractive candidate for development.



PNU-97333

1



PNU-141962

5

Conversion of PNU-97333 to PNU-141962 appears to be a simple transformation on paper,the chemical reduction of amides being well documented.[2] However, the required reduction of the secondary amide must be achieved without reduction of the tertiary amide at C-18 or interference from the sensitive vinylogous ether centered at C-24. There is some literature precedent[3] for the selective reduction of tertiary amides in the presence of secondary amides. However, to the best of our knowledge, when this work began, the desired selective reduction of a secondary amide in the presence of a tertiary amide had not yet been reported. There is significant literature precedent[5−8] for the reduction of carbamate-protected lactams to their corresponding hemiaminals. Hemiaminals, in a one-pot multistep process (hydrogenolytic deprotection, decarboxylation, dehydration, then hydrogenation over Pd/C) provide the desired secondary amine.[9] Therefore, activation of the secondary amide carbonyl by conversion to the carbamate appeared to be the key to the transformation. In fact, a preliminary application of this approach was recently reported by Lee and Clothier.[4]

Results and Discussion

Our objective was to develop an efficient route to PNU-141962 that avoided chromatographic purification, eliminated the use of toxic and highly flammable solvents, and was scalable. Our colleagues in Pharmacia Animal Health demonstrated that direct reductions of PNU-97333 were indeed possible, but their laboratory procedures were not amenable to scale-up. The highest-yielding direct-reduction procedure was run in aqueous glyme at reflux. It utilized an excess of sodium bis(2-methoxyethoxy)aluminum hydride (Vitride) and a portion-wise addition of 60 equiv of lithium borohydride. Although we were able to use this procedure to provide a small sample of PNU-141962 in 30% yield for reference and characterization, our efforts quickly turned to what we hoped would be an efficient multistep approach that involved activation of the secondary amide as the urethane. Animal Health chemists were pursuing similar methodology at the time and eventually developed a four-step sequence (Scheme 1) which enabled them to prepare 25 g of PNU-141962.

Development of this approach continued as bulk-drug requests escalated; we began by replacing the expensive FMOC-protecting group with the affordable ethyl carbamate. The urethane was then reduced sequentially by treatment with Vitride followed by sodium borohydride in dioxane or glyme (Scheme 2). Unfortunately, this method produced significant quantities of the difficult-to-separate ring-opened byproduct

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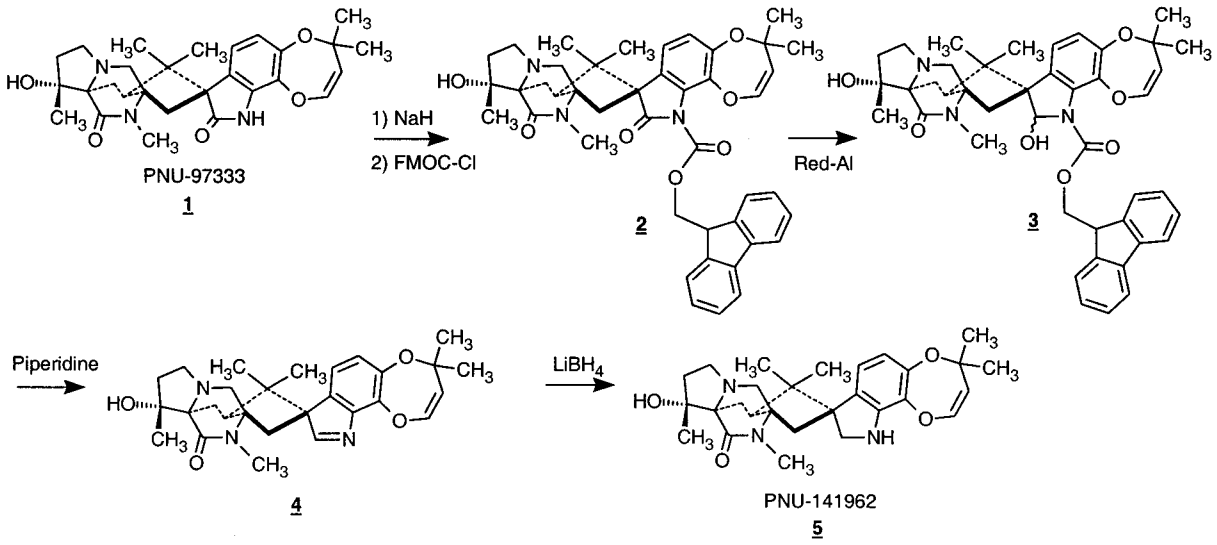
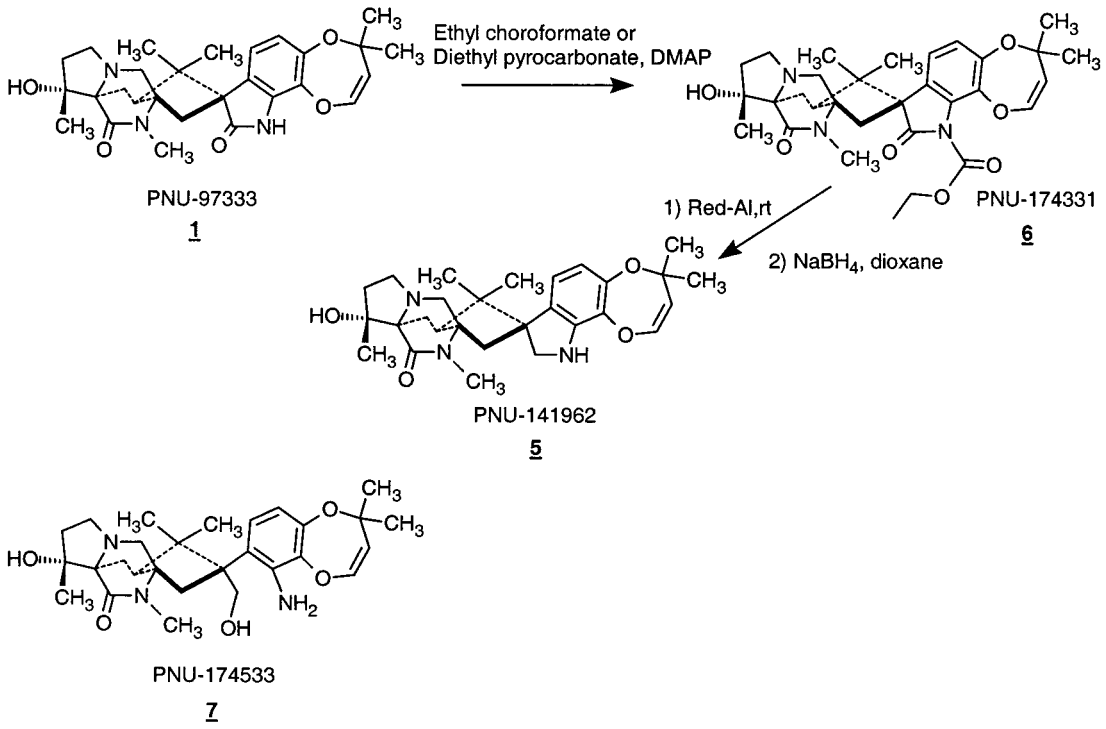
Scheme 1



Scheme 2



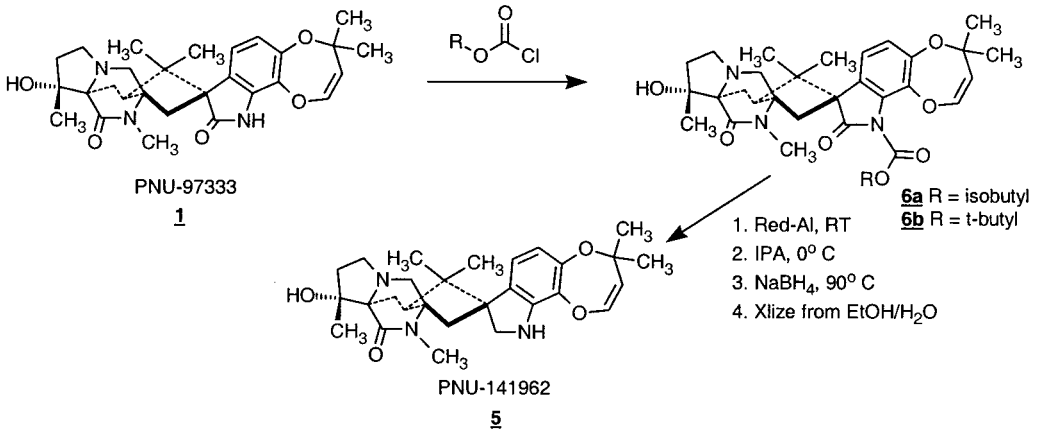
PNU-174533 (***7***), in addition to providing unacceptable levels of the toxic active PNU-97333 (***1***) (Scheme 2).

This caused the major drawback of the sequences depicted in Schemes 1 and 2, their reliance on chromatography as the final purification method for PNU-141962. Clearly, for such an approach to succeed in a process chemistry setting, a crystallization or precipitation method for final product isolation had to be developed. It was quickly determined that PNU-141962 could be significantly upgraded via crystallization from ethyl acetate or ethyl acetate/heptane mixtures; however, the impurities spectrum that were found clearly taxed this method (vide supra).

In addition to the variable and unacceptable product mixtures obtained using the ethyl carbamate method of Scheme 2, another more serious problem was the safety issue concerning the use of thermally labile ethyl chloroformate. Therefore, we chose to activate with the readily available isobutyl chloroformate (Scheme 3). We were able to quickly prepare several hundred grams of the requisite isobutyl carbamate intermediate in the lab. On small scale, with less than 10 g input, the double-reduction procedure performed well. *It is interesting to note, however, that the isopropyl alcohol quench to generate the presumed hydroxyurethane intermediate* ***3***[10] *from the aluminate is an absolute requirement for the success of the overall reduction.* In the absence of this alcohol quench, the intermediate is completely inert to borohydride treatment. Despite these early successes, the procedure was not robust on even modest

(10) Attempted characterization of this material was inconclusive, but suggestive of the assigned structure.

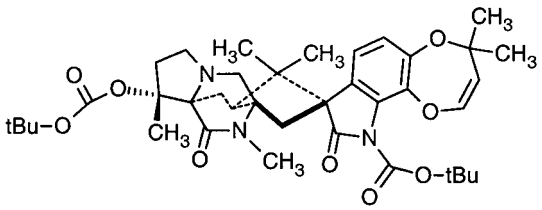
Scheme 3



laboratory scale; the PNU-141962 produced was contaminated with 4−6% PNU-97333, and our recrystallization procedure was ineffective. Although material of sufficient purity could be obtained after repeated recrystallizations, recoveries were poor, and a chromatographic purification became our only purification option on even this modest scale.

It was determined that the "residual" PNU-97333 was in fact produced during the course of the reduction, and was not unreacted starting material. It apparently results from a nonselective reduction at the undesired urethane carbonyl, and although this reductive pathway is greatly disfavored over the desired alternative, apparently even a 20:1 selectivity could not be tolerated by our purification scheme. Noting that reduction of the isobutyl carbamate was significantly more selective than in the ethyl series, we surmised that increased steric congestion near this carbonyl might reduce the undesired reduction pathway. Thus, we prepared the *tert*-butyl urethane, the reduction of which produced the desired outcome. In this fashion, we were able to limit partitioning via the wrong reductive pathway to less than 1%. The activating-group change was made in conjunction with a solvent switch to methylene chloride from THF for the urethane-formation reaction. This proved to be highly beneficial and provided much cleaner phase breaks during the aqueous partition to remove the (dimethylamino)pyridine catalyst and hydrolyzed di-*tert*-butyldicarbonate byproduct, *tert*-butyl alcohol. The separations were slow, due to the presence of the *tert*-butyl alcohol generated, but efficient. Our crystallization method, while not ideal, did provide the desired upgrading at this level of purity.

With the overall sequence now well in hand, we quickly moved into 200-L vessels in the pilot plant. The first of our planned two 5-kg-scale protections went as expected, and the isolated chemical yield was 84%. We had some operational difficulties during the second pilot run. We were not able to isolate the product in a timely fashion, and our first crop yield was only 65%. Unfortunately, a second crop was contaminated with 7.5% of the over-reaction product **8**, normally seen only as a minor impurity. Typically, there was only 1−2% of this carbonate present in the crude reaction mixture, and it is handily eliminated using our crystallization method. Although it may be possible to selectively hydrolyze the carbonate or even reduce it directly, time pressures forced us to move on and simply accept the loss.



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The double reduction conducted on the *tert*-butyl carbamate went very well and scaled uneventfully into 12-L equipment in the lab. The PNU-141962 produced was of extremely high chemical purity, but unfortunately retained 0.9% toluene. Early small lots of bulk active had been isolated by a knockout procedure that involved a toluene chase with either isooctane or heptane. This previous experience led us to believe that residual solvent would not be an issue with this compound. Although we eventually found that refluxing heptane at high dilution would reduce toluene levels, this technique was unacceptable on scale. A mixture of 15% ethyl acetate in heptane at temperatures that were more workable and at more reasonable dilutions was successful, but only on gram scale.

The tenacity with which PNU-141962 held solvent made us redirect our approach; rather than try to eliminate the residual solvent, we decided instead to choose it. From a toxicity standpoint, residual water is obviously the ideal choice, and ethanol is arguably a close second. A 60/40 water:ethanol mixture at 60° C proved to be an ideal recrystallization solvent. Not only did this method allow us to reduce residual toluene to less than 80 ppm from existing lots of PNU-141962, but incorporation of an ethanol chase into the final isolation process also allowed us to directly precipitate acceptable-quality PNU-141962.

Now that all the purity pitfalls had been demonstrated to be avoidable on large lab scale, we readied our reduction process for piloting. The only issue we foresaw involved the workup. In the process, the second reduction was completed by transferring the hemianimal product of the Vitride reduction into a slurry of 10 equiv of sodium borohydride in toluene. The slurry was then heated to 95°

C. In the laboratory, the workup involved quenching with water followed by filtration through Celite. Since basic aqueous solutions of sodium borohydride are reasonably stable, the excess borohydride was not completely quenched by this treatment. Not only does water precipitate the boronates that are then collected in the cake, it also precipitated unreacted sodium borohydride, a definite safety concern for pilot plant operations. Typically borohydride reductions can be completely quenched by addition of acidic aqueous media, but we were concerned about hydrolysis of the sensitive vinylic ether linkage. We explored unsuccessfully a number of quenches that would leave the vinylic ether intact. Although sodium borohydride is very soluble in 10−20% aqueous sodium hydroxide, without an agitated filter it would not be possible to dissolve the residual borohydride from the cake. For the modest pilot-plant scale reactions conducted, the filtration was accomplished on a Nutsche filter coated with Celite. The cake was then dried under nitrogen and packaged as hazardous waste for disposal. Obviously, this waste issue must be addressed if this process is to be conducted on a significantly larger scale.

The double reduction was successfully conducted in duplicate 4-kg runs. The first produced 2.3 kg PNU-141962 (70%) and with fine-tuning of the ethanol/water ratio the second gave 2.66 kg (81%). Both lots met all specifications and were released.

Conclusions

A route was successfully developed and demonstrated on scale to selectively reduce a secondary amide in the presence of a tertiary amide. The key to the success of the approach was to activate the secondary amide as a urethane. The urethane was reduced with Vitride and quenched with 2-propanol, and the overall transformation was finished with sodium borohydride. Steric factors were shown to influence the reduction pathway. The method allowed for the production of 4.9 kg of PNU-141962 under GMP conditions. The yields were good to excellent, no chromatographies were required, and the procedure is readily amenable to further scale-up. Obvious places for future process improvement are in the isolation of active sodium borohydride (which must be used in molar excess) and its disposal.

Experimental Section

General. Reagents were obtained from Aldrich Chemical Co. and were used without further purification. The Vitride was obtained from Zeeland Chemicals, Inc. and used as received. Purified solvents were obtained from EM Science and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz on $CDCl_3$ solutions.

Preparation of PNU-143982 (2). A clean, dry 200-L reaction vessel under an inert atmosphere was charged with 4.66 kg (9.44 mol) of PNU-97333, 127 g (1.03 mol) of 4-(dimethylamino)pyridine, and 50 L of methylene chloride. Di-*tert*-butyldicarbonate (4.6 kg, 21.1 mol) and 25 L of methylene chloride were charged to a separate vessel. The di-*tert*-butyldicarbonate was stirred until it dissolved and was then transferred into the reaction vessel containing the substrate. The reaction mixture was stirred a minimum of 8 h or until PNU-97333 could no longer be detected by HPLC.[11] A saturated sodium chloride solution (60 kg) and 50 kg of 0.1 M hydrochloric acid solution were added, and the reaction was stirred and then allowed to separate. The aqueous phase was extracted with 3 × 22 L of methylene chloride. The combined organic extracts were washed with 15 kg of saturated sodium bicarbonate solution and then 30 kg of saturated sodium chloride solution. The methylene chloride was swapped for 66 L of *n*-heptane via an atmospheric distillation. The distillation was continued to final volume of 65 L and an internal pot temperature of 70−72 °C. The mixture was then cooled to LT −15 °C for a minimum of 3 h, filtered, and rinsed with heptane. The product PNU-143982 was dried with single-pass 50 °C nitrogen. The yield was 4.66 kg (84%). ^{1}HNMR δ 6.82 (s, 2H), 6.28−6.30 (d, 1H), 4.85−4.87 (d, 1H), 3.58−3.61 (d, 1H), 3.1 (m, 1H), 3.06 (s, 3H), 2.89 (t, 1H), 2.74−2.78 (d, 1H), 2.63 (s,1H), 2.55−2.58 (d, 1H), 2.33 (m, 1H), 2.22 (m, 1H), 1.83 (m, 5H), 1.64 (s, 3H), 1.59 (s, 9H), 1.46 (s, 3H), 1.42 (s, 3H), 1.03 (s, 3H), 0.69 (s, 3H); ^{13}CNMR δ 179.86, 171.29, 148.72, 147.59, 138.87, 138.62, 130.93, 124.89, 120.09, 119.26, 115.75, 84.71, 79.80, 78.07, 71.19, 65.25, 63.11, 59.21, 51.96, 51.35, 47.53, 38.09, 37.18, 29.95, 29.56, 27.70, 25.92, 23.71, 22.20, 20.37, 19.09.

Preparation of PNU-141962(4). A clean, dry 200-L reaction vessel under an inert atmosphere was charged with 4.1 kg (6.90 mol) of PNU-143982 and 90 L of toluene and was cooled to between −5 and 5 °C. Vitride 70%, 5.99 kg (20.7 mol) was diluted with 5 L of toluene and was slowly added to the substrate solution, keeping the temperature between 0 and 10 °C. Thirty minutes after the addition was complete, the reaction was shown to be complete by HPLC analysis.[11] It was then quenched by the cautious addition of 13.3 L of isopropyl alcohol. A clean, dry 400-L vessel under an inert atmosphere was charged with 2.62 kg of sodium borohydride (69.0 mol), and then the toluene solution of the intermediate product was added. The reaction mixture was heated to 90−95 °C for 18−24 h, and was monitored by HPLC.[11] It was cooled to 20−30 °C, and 4.0 kg of water was added. After stirring for a minimum of 1 h, the reaction mixture was filtered through Celite, and the cake was rinsed with 2 × 10 L of toluene. The filtrate was concentrated in vacuo to about 30 L, and 163 kg of absolute ethanol was added. It was concentrated again to a volume of 30 L, cooled to 20−30 °C, and 50 kg of water was added. It was then cooled to 5 °C for 8−10 h, filtered, and washed with 5 L of cold water. The PNU-141962 was dried with single-pass 40 °C nitrogen to a constant weight of 2.66 kg (81% yield).

(11) HPLC conditions: Solid samples were made up by dissolving the desired material in mobile phase at about 1.0 mg/mL concentration. Reaction mixtures were diluted directly with mobile phase to about 1.0 mg/mL concentration. Column: Hypersil C-18 BDS column. Injection volume:10 μL. Mobile phase: acetonitrile:0.1 M ammonium chloride = 1:1. Flow rate:1.0 mL/min. Detector: 230 nm. Retention times: (1) PNU-97333 4.8−5.1 min, (2) PNU-143982 16.6−17.0 min, (3) PNU-141962 7.3−7.7 min.

^{1}HNMR δ 6.69−6.67 (d, 1H), 6.41−6.39 (d, 1H), 6.30−6.28 (d, 1H), 4.80−4.78 (d, 1H), 3.51−3.48 (d, 1H), 3.39−3.37 (d, 1H), 3.17 (m, 1H), 2.54−2.51 (d, 1H), 2.22 (m, 4H), 2.70 (t, 1H), 1.86 (m, 3H), 1.76 (t, 1H), 1.64 (s, 3H), 1.42 (s, 6H), 0.88 (s, 3H), 0.85 (s, 3H); ^{13}CNMR δ 172.44, 145.89, 143.39, 139.37, 136.70, 126.64, 119.68, 114.51, 113.83, 79.30, 78.00, 71.53, 64.31, 61.05, 60.54, 58.17, 54.80, 51.57, 45.33, 41.80, 38.16, 29.95, 29.92, 25.58, 22.92, 22.50, 19.18, 19.09. Anal. Calcd For $C_{28}H_{37}N_3O_4$: C, 70.12; H, 7.78; N, 8.76. Found C, 70.06; H, 7.82; N, 8.73.

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