Simultaneous Hydrogenolysis of p-Nitrobenzyl Esters and Carbamates Side-Chains in the THF 1β-Carbapenem OCA-983 in Biphasic Media

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Abstract: Deprotection of p-nitrobenzyl esters and valyl carbamates in carbapenem CL 192,276 produced the active compound OCA-983 in excellent yields. Straight chain alkanols such as 1-butanol, 1-pentanol and 1-hexanol in water at certain ratios were effective solvent systems. Alkyl acetates in water also resulted in simultaneous deprotection of PNB and PNZ side-chains albeit at slower rates. The deprotected carbapenem was isolated in excellent yield and purity after removal of the aqueous media. This procedure is applicable to sensitive compounds that are soluable in water without the need to use a buffer and allows for ease of isolation from the aqueous phase.

Key Words: OCA-983, carbapenems, deprotection, PNB/PNZ side-chains, biphasic media, n-butanol-H_2O, ethyl acetate-H_2O.

INTRODUCTION

Resistance to antimicrobial agents is a medical problem of growing concern with global impact. Strategies to overcome this problem relied on investigations targeted towards new classes of antibiotics or combinations of β-lactam antibiotics with β-lactamase inhibitors. The latter class of inhibitors are designed to overcome resistance to β-lactam antibiotics particularly in Gram-negative bacteria. Despite good advances made to date, more time is needed to reveal the success of the current strategies. Another strategy of valuable impact concerns the more prudent use of existing antibiotics [1]. Tygacil® is a representative of glyclcyclines class of tetracyclines that has recently been indicated for the treatment of both hospital- and community- acquired complicated intra-abdominal infections and complicated skin and skin structure infections in adults.

In the cases of carbapenems and penems, clinically used agents are effective only when administered by the parental route. Pharmacokinetic advantages such as oral activity and longer half-life together with broad spectrum of antimicrobial activity are desirable characteristics in the next generation of antibiotics from these classes. A recent review addressed the advantages of orally active carbapenems including esters and amino acids peptides of five agents at various stages of development (GV-118819, GC-834, L-084, DZ-2640 and CL 191,121) [2]. Despite the slow development or even the termination in development of some agents, useful information is provided in this review particularly on pharmacokinetic parameters in relation to stability in the stomach and intestine. To date, very few oral β-lactam antibiotics have emerged. An interesting matter regarding oral activity, relative ease of availability and the rate of emergence of resistance particularly in Gram-negative bacteria is a relevant issue for consideration but is beyond the scope of this manuscript.

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A distinguishing aspect of the current β-lactam antibiotics with oral activity is the presence of an ester moiety that requires hydrolysis to active drug Fig. (**1**). The pro-drug faropenem daloxate is hydrolyzed in plasma to faropenem Scheme (**1**). Target binding affinities to penicillin binding proteins (PBPs) suggest strong binding to high molecular weight PBPs from Gram-negative and Gram-positive bacteria which translated to potent antibacterial activity in these species. However, faropenem demonstrated weak activity in comparison to parental carbapenems such as imipenem and meropenem against *Pseudomonas aeruginosa* due to limited uptake and active efflux mechanisms, while its bactericidal activity against *S. pneumonine* and *H. influenzae* was good. On these grounds faropenem was recently selected for clinical development in the treatment of community-acquired respiratory tract infections [3].

Tebipenem (previously known as L-084) is a new oral carbapenem which *in vitro* has demonstrated potent and broad-spectrum activity against microorganisms that cause respiratory tract infections and urinary tract infections. Phase II clinical studies in adult patients are currently in progress [4].

OCA-983 is a THF-1β-methylcarbapenem with potent activity against Gram-positive and Gram-negative aerobes and anaerobes including clinical isolates. It possesses rapid bacteriacidal activity [5]. A distinguishing structural feature between OCA-983 and tebipenem is that OCA-983 is a L-valyl/peptidic pro-drug of CL 191,121 whereas L-084 is the pivaloyloxymethyl ester of L-036. Both OCA-983 and L-084 are hydrolyzed in serum and *in vivo* to the active compounds CL 191,121 and TBM (L-036) respectively, Scheme (**1**). In this manuscript we report on the simultaneous removal of PNB and PNZ ester and carbamate protecting groups using a novel biphasic system. These conditions are suitable for sensitive compounds that are soluble in aqueous media.

METHODS AND MATERIAL

GENERAL METHODS

^{1}H NMR spectra were determined with s Bruker DPX-400 spectrometer at 400 MHz. Chemical shifts are reported

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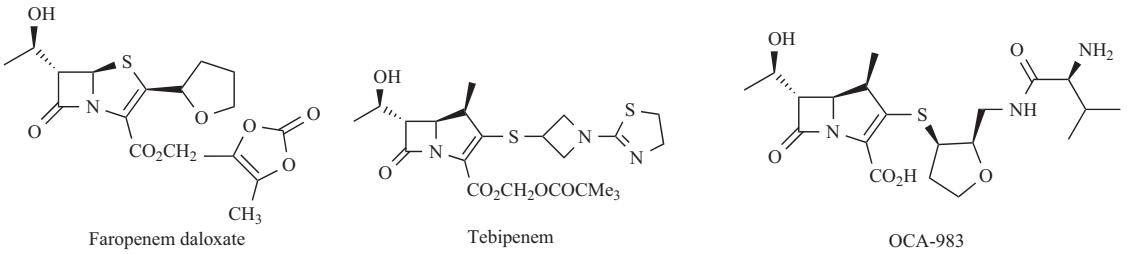
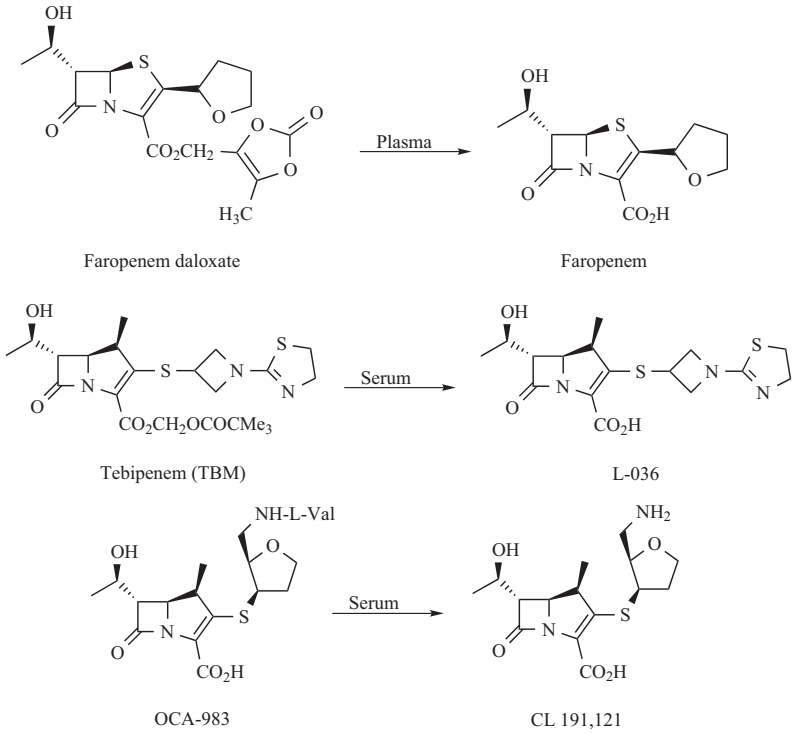


Fig. (1). Structures of orally active compounds.



Scheme (1). Prodrugs of orally active penems and carbapenems.

in parts per million (δ) relative to residual chloroform (7.26 ppm), TMS (0 ppm), or dimethylsulfoxide (2.49 ppm) as an internal reference with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Electrospray (ES) mass spectra were recorded in positive or negative mode on a Micromass Platform spectrometer. Electron impact and high-resolution mass spectra were obtained on a Finnigen MAT-90 spectrometer. Optical rotations were measured at 25°C in water using Jasco DIP 370 model. Reactions were monitored by analytical high pressure liquid chromatography (HPLC) using the following conditions:

Column: YMC-Pack ODS-AM, S-3 micron, 150 x 4.6 mm

Mobile Phase: Gradient A = water; B= acetonitrile

Time(min)	%A	%B
0	98	2
5	98	2
25	75	25
30	0	100

Flow rate: 1.0 mL/minute

Temperature: 40°C

Detection: DAD 220-300 nm

Injection solvent: acetonitrile/water; 10 microliter injection; HP1090

The terms “concentrated” and “evaporated” refer to removal of solvents using a rotary evaporator at water

aspirator pressure with a bath temperature equal to or less than 40°C. Unless otherwise noted, reagents were obtained from commercial sources and were used without further purification.

EXAMPLE 1: 1-butanol/water (4R, 5S, 6S)-3-[[(2R,3R)-2-[[[(S)-2-Amino-3-methyl-1-oxobutyl]amino]methyl] tetrahydro-3-furanyl]thio]-6-[(R)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

A mixture of 4-nitrobenzyl (4R,5S,6S)-6-[(1R)-1-hydroxyethyl]-4-methyl-3-{[(2R,3R)-2-({[(2S)-3-methyl-2-({[(4-nitrobenzyl)oxy]carbonyl}amino)-butanoyl]amino}methyl) tetrahydrofuran-3-yl]thio}-7-oxo-l-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (0.200 g), 50% wet 10% palladium on carbon (0.256 g), 1-butanol (20 mL) and water (20 mL) is hydrogenated in a Parr apparatus at 56 psi for 8 hours. The catalyst is then removed by filtration through diatomaceous earth and washed with 2x6 mL of water. The 1-butanol layer is extracted once with 6 mL of water. The combined aqueous solution (ca. 38 mL) is extracted twice with 2x10 mL of isopropyl acetate, concentrated under reduced pressure below 28 °C to ca. 20 mL and lyophilized and give 97.1 mg (83%) of the desired product as a slightly tan solid. ^{1}H NMR(D_2O) Λ 4.1-4.17 (m, 3H); 3.96 (q, 1H); 3.78 (m, 2H); 3.58 (d,1H, *J*=6.0 Hz); 3.2-3.4 (m, 4H); 2.34 (m, 1H); 2.0-2.1 (m, 2H); 1.27 (d, 3H, *J*=6.3 Hz); 1.18 (d, 3H, *J*=7.2 Hz); 0.99 (d, 6H, *J*=6.9 Hz) MS (ES), m/z: 442.3 $(M+H)^+$; rotation $[a]_D25$ +42±1° (c 0.84, H_2O); purity by analytical HPLC (100%).

EXAMPLE 2: 1-hexanol/water (4R, 5S, 6S)-3-[[(2R,3R)-2-[[[(S)-2-Amino-3-methyl-l-oxobutyl]amino]methyl]tetrahydro-3-furanyl]thio]-6-[(R)-1-hydroxyethyl]-4- methyl-7-oxo-l-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

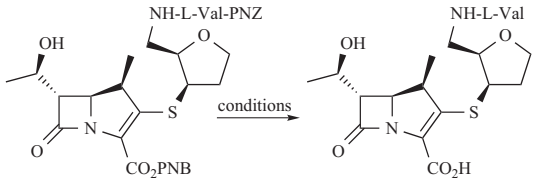
To a mixture of 0.302 g of 4-nitrobenzyl (4R,5S,6S)-6-[(lR)-1-hydroxyethyl]-4-methyl-3-{[(2R,3R)-2-({[(2S)-3-methyl-2-({[(4-nitrobenzyl)oxy]carbonyl}amino)-butanoyl] amino}methyl)tetrahydrofuran-3-yl]thio}-7-oxo-1-azabicyc1o [3.2.0]hept-2-ene-2-carboxylate in 30 mL of 1-hexanol is added 30 mL of water followed by 0.15 g of 10% palladium-on-carbon. The reaction mixture is then hydrogenated in a Parr apparatus at 54 psi for 23 hours. To the mixture is added 30 mL of water and the mixture is filtered through a pad of diatomaceous earth. The pad is washed with an additiona1 20 mL of water. The filtrate is transferred to a separatory funnel, the aqueous layer separated and the 1-hexanol layer is washed with 10 mL of water. The combined aqueous layers are washed with 100 mL of ethyl acetate and lyophilized to afford 0.144 g (82%) of the desired product. MS (ES), m/z:442.3 $(M+H)^+$, 99.5% area percent pure by analytical HPLC.

EXAMPLE 3: ethyl acetate/water (4R, 5S, 6S)-3-[[(2R,3R) 2-[[[(S)-2-Amino-3-methyl-1-oxobutyl]amino]methyl] tetrahydro-3-furanyl]thio]-6-[(R)-1-hydroxyethyl]-4-methyl-7-oxo-l-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

To a mixture of 5.064 g of 4-nitrobenzyl (4R,5S,6S)-6-[(lR)-1-hydroxyethyl]-4-methyl-3-{[(2R,3R)-2-({[(2S)-3-methyl-2-({[(4-nitrobenzyl)oxy]carbonyl}amino)-butanoyl] amino}methyl)tetrahydrofuran-3-yl]thio}-7-oxo-l-azabicyclo [3.2.0]hept-2-ene-2-carboxylate in 120 mL of ethyl acetate is added 5 mL of water, (about a 4% water: ethyl acetate biphasic solvent mixture) and after standing for about 5 minutes, gives a clear biphasic solvent mixture. An additional 55 mL of water is added followed by 2.5 g of 10% palladium-on-carbon. The reaction mixture is then hydrogenated in a Parr apparatus at 54 psi for 20 hours. The mixture is transferred to a separatory funnel and 100 mL of water is added. The aqueous layer is separated and the ethyl acetate layer washed with 20 mL of water. The combined aqueous layers are filtered through a pad of diatomaceous earth and the pad washed with an additional 30 mL of water. The combined aqueous layers are washed with 200 mL of ethyl acetate and lyophilized to afford 2.3 g (78%) of the desired product. MS (ES), m/z:442.3 $(M+H)^+$, 98.9% area percent pure by analytical HPLC.

RESULTS AND DISCUSSION

The penultimate step in the synthesis of OCA-983 is the deprotection of the PNB ester and the carbamate PNZ groups by using H_2 over a catalyst 10% Pd/C. This step proceeds in low yields due to the generation of multiple products including β-lactam ring opening side products Scheme (**2**). In this manuscript we describe our conditions to deprotect both PNB and PNZ carbamate side-chains concomitantly. Since the rates of deprotecting the PNB ester and the PNZ carbamates are likely to be different, reaction conditions are needed to drive this reaction to completion so that purification of OCA-983 is facilitated. Over-hydrogenation is a factor for consideration since the β-lactam moiety is prone to ring opening. The concept of exploring a biphasic system for this transformation appeared to be attractive because of the favorable solubility of OCA-983 in the aqueous phase. Moreover, the organic products formed from PNB are soluble in the organic phase and therefore can be separated out. Fig. (**2**) illustrates side products expected from this transformation.



CL 192,276 OCA-983

Conditions: *t*-BuOH: H_2O, H_2 56 psi 10%Pd/C 8 h, yield 79-85% HPLC> 98%; EtOAc: H_2O, H_2 55 psi 10% Pd/C 17 h yield 79-90% HPLC > 98%;1-hexanol: H_2O, H_2 54 psi 10%Pd/C 23 h, yield 79% HPLC > 98%

Scheme (2). Hydrogenolysis conditions leading to OCA-983.

Our initial attempts to achieve this goal relied on changing solvents and reaction time. In these attempts isolating the desired product was problematic due to the difficulties in controlling the hydrogenolysis step reproducibly and due to issues of purifying the desired product. To circumvent further hydrolysis of the β-lactam containing compound a buffer was used. This however, proved to be a problematic due to the difficulty of removal of the buffer from OCA-983 without substantial decomposition. We explored the concept of removing both protecting groups in a biphasic solvent mixture having an organic solvent using

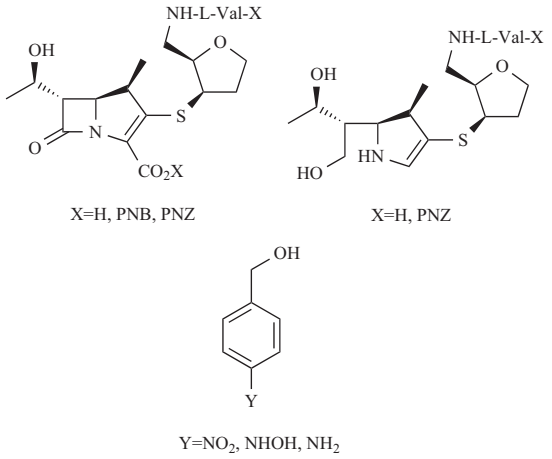


Fig. (2). Structure of by-products.

Pd/C as a catalyst under neutral conditions. Eliminating the use of aqueous buffers to neutralize the carbon dioxide formed from the simultaneous reductive step of removing PNB and PNZ esters proved to be advantageous. The organic layer containing the aromatic impurities is separated out while the aqueous layer containing the desired compound is subjected to lypolization or reverse osmosis to remove water. Table (**1**) lists promising results obtained with alkyl acetates/H_2O under various conditions. Importantly, the purity of the isolated product as determined by HPLC indicated the potential value of this approach to similar synthetic challenges. It also seems that in this system the longer reaction times of 22h are advantageous in ensuring complete hydrogenation of all impurities. Shortening, reaction time was achieved satisfactorily using 1-butanol, 1-pentanol or 1-hexanol/H_2O mixtures. Indeed in these cases both the amount of catalyst and the reaction time could be reduced (Table **2**). The use of the above conditions allows the preparation of OCA-983 in 78-83% overall isolated yields.

The PNB and PNZ protecting groups are widely used in β-lactam [6,7] and peptide chemistry [8,9]. New deprotection conditions using Zn in buffer (PH 6.5) have been recently reported [10]. The biphasic system advantage [11] presented in this work compliments earlier methods and provides an alternative in cases where the final compound is water soluble and sensitive to decomposition. Importantly, this procedure eliminates the use of a buffer which facilitates the purification steps substantially.

Table 1. Conditions for Synthesis of OCA-983 Using Alkyl Acetates-H_2O Mixtures in 10% Pd/C

Entry	Solvent (mL)	Reaction Condition Time (hr), Pressure (psi)	Yield[a] (%)/HPLC Purity (%)
1	$CH_3CO_2C_2H_5$ (20)/H_2O (20)	22,56	71, 99
2	CH_3CO_2n-C_3H_7 (20)/H_2O (20)	22,56	74, 99
3	$CH_3CO_2C_3H_7$ (20)/H_2O (20)	22,56	69, 99
4	CH_3CO_2i-C_3H_7 (30)/H_2O (30)	6.5,55	66, 94
5	CH_3CO_2i-C_3H_7 (10)/n-C_4H_9OH (10/H_2O (20)	2,45	44, 98
6	$CH_3CO_2C_2H_5$ (25)/n-C_4H_9OH (25)/H_2O (50)	8,56	74, 99

[a]Yields from experiments with 0.2 g of starting material and 0.1g of catalyst

Table 2. Conditions for the Synthesis of OCA-983 Using Alcohol – H_2O Mixtures

Entry	Solvent (mL)	Catalyst	Reaction Condition Time (hr), Pressure (psi)	Yield[a] (%)/HPLC Purity (%)
1	n-C_4H_9OH (5)/H_2O (10)	10% Pd/C	1.5,45	Emulsion
2	n-C_4H_9OH (20)/H_2O (20)	20% $Pd(OH)_2/C$[b]	6.5,55	85, 99
3	n-C_4H_9OH (20)/H_2O (20)	20% $Pd(OH)_2/C$[c]	8,55	85, 99
4	n-$C_5H_{11}OH$ (20)/H_2O (20)	10% Pd/C	20,55	74, 95
5	n-$C_6H_{13}OH$ (30)/H_2O (30)	10% Pd/C	23,54	82, 99
6	C_2H_5OH (30)	10% Pd/C	4,45	43

[a]Yields from experiments with 0.2 g of starting material and 0.1 g of catalyst. [b] 0.05 g of catalyst. [c] 0.064 g of catalyst

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