Synthesis of Platensimycin Aromatic Fragment

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ABSTRACT

The aromatic fragment of Platensimycin was synthesised in a protected form (2,4-dimethoxy-3-aminomethylbenzoate) to facilitate the amide coupling to the pentacyclic ketolide fragment. The proposed reaction scheme consists of a Lieben haloform reaction as a key step to introduce the carboxylic acid functional group into the target molecule. Improvement to our 1st generation synthetic route gave an overall yield of 55%, which was comparable to other literature reports. The modified Lieben haloform method used to convert a methyl ketone directly to an ester has applications to produce esters or amides by changing the nucleophile. Other schemes to synthesise the protected form of the aromatic fragment, for example, using Baeyer-Villiger oxidation as a key step were also investigated.

INTRODUCTION

Antibiotics work primarily by preventing the growth or replication of a single bacterium cell. The most common mechanisms of action include inhibiting bacterial cell wall biosynthesis, blocking bacterial protein synthesis and blocking DNA replication and repair. Bacterial pathogens can become resistant to antibiotics through mechanisms such as inactivation of the antibiotics, active export or efflux of antibiotics such that therapeutic concentrations are not attained in the bacterial cytoplasm, and modification of the targets of antibiotics and rendering them insensitive while retaining its essential cellular function.

Antibiotic resistance is favored when the bacteria is exposed to an environment with a selection pressure against it. The more the bacteria are exposed to a certain antibiotic, the faster it would develop resistance towards it. Hence, antibiotic resistance of a certain species of bacteria is only a matter of time, resulting in a constant need of new antibiotic discovery and development while trying to impede the emergence of antibiotic resistance.¹ As such, a new class of antibiotics, preferably with a new mechanism of action apart from just structural difference, would indeed contribute to these efforts.

Platensimycin shows strong broad-spectrum Gram-positive antibacterial activity via the selective inhibition of fatty-acid biosynthesis in bacteria via blocking the active site of FabF enzyme. This enzyme catalyses the elongation of the growing fatty acid chain by adding acetate units to form a β-ketoacyl substrate.²

It has been shown that Platensimycin is the selective inhibitor for the FabF condensing enzymes, from the correspondence between MICs and IC₅₀ values for the inhibition of cellular lipid biosynthesis, and displays broad-spectrum activity, in vivo efficacy and no observed toxicity in mice model. It does not inhibit DNA, RNA, protein or cell wall biosynthesis which the current major classes of antibiotics discussed previously worked. By virtue of this unique mode of action by Platensimycin, it shows no cross-resistance to

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other main antibiotic-resistant strains such as methicillin- and vancomycin-resistant \textit{S. aureus} or \textit{enterococci}.\textsuperscript{[3]} Thus, this antibiotic may provide part of the solution to the widespread problem of MRSA worldwide.

Platensimycin’s unique mode of action which acts through new mechanisms are attractive as they offer the prospect of effectively combating infectious resistant to all current drugs.\textsuperscript{[4]} The unique aromatic fragment contributes to the strong binding of the natural product within the active site of FabF through specific interactions with four highly conserved amino acid residues, i.e. H303, H340, C163 and F400, that are critical to the enzyme’s catalytic activity. Furthermore, the multiple van der Waals interactions between Platensimycin and the enzyme’s critical amino acid residues would suggest that drug resistance due to mutation of the enzyme’s active site would be less likely.\textsuperscript{[3]}

The aim of this project is to pursue a synthesis of the uncommon 2,4-dihydroxy-3-aminobenzoic acid fragment of Platensimycin. This fragment will be synthesized as the protected form to facilitate the amide coupling to the pentacyclic ketolide fragment. A total synthesis of Platensimycin has been published by Nicolaou \textsuperscript{[4]}, together with several formal synthesis of the pentacyclic ketolide fragment by other groups.\textsuperscript{[5,6]} However this project will serve to suggest another plausible synthetic approach towards the aromatic fragment of Platensimycin, which is part of the effort towards the total synthesis of Platensimycin by Lear Group.

MATERIALS AND METHODS

\textbf{Improved 1\textsuperscript{st} Generation Synthetic Route (with Lieben haloform reaction as a key step)}

\textbf{2,4-dihydroxy-3-nitroacetophenone (5)}
Polyphosphoric acid (PPA) was heated to about 50°C. When it became less sticky, acetic acid (506 mg, 8.47 mmol, 2.36 equiv.) and 2-nitroresorcinol (550 mg, 3.58 mmol, 1 equiv.) were added. After the reaction mixture was stirred for 2h at a temperature around 70-80°C, the reaction was quenched by addition of cold water (165 ml). The reaction mixture was extracted with ethyl acetate (5 x 30 ml). The combined organic layers were dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and the solvent was removed under reduced pressure at 35°C. The crude product was purified by silica gel column chromatography using dichloromethane as eluent. 61% yield was obtained as yellow crystalline solid.

\textbf{2,4-dimethoxy-3-nitroacetophenone (6)}

5 (450 mg, 2.30 mmol, 1 equiv.) and anhydrous K\textsubscript{2}CO\textsubscript{3} (2.5373 g, 18.36 mmol, 8 equiv.) was stirred in acetone (23 ml) at room temperature. MeI (5.2121g, 36.72 mmol, 16 equiv.) was added dropwise to the stirring mixture. The reaction mixture was then heated under gentle reflux for 6h. After cooling, the mixture was filtered and the filtrate evaporated under reduced pressure to give the crude product. The crude product was purified by silica gel column chromatography using 30% ethyl acetate/hexane as eluent. 95% yield was obtained as pale yellow oil.

\textbf{Direct synthesis of (7) from (6) using \textit{tert}-butyl hypochlorite in alkaline methanol}

\textbf{Preparation of \textit{tert}-butyl hypochlorite}\textsuperscript{[7]}

14% sodium hypochlorite (23 ml) was placed in a conical flask, wrapped with aluminum foil, immersed in an ice-bath and rapidly stirred. A solution of \textit{tert}-butyl alcohol (5 ml) and glacial acetic acid (3 ml) was added in a single portion to the hypochlorite solution,
and the stirring was continued for 3 minutes. The reaction mixture was then transferred to a separatory funnel and the lower aqueous layer was discarded. The yellow organic layer was washed with saturated sodium bicarbonate (10 ml) followed by deionised water (10ml). The organic layer was then dried over anhydrous Na₂SO₄, filtered and used in the following reaction immediately.

6 (50 mg, 0.22 mmol, 1 equiv.) was dissolved in dry methanol (8 ml) and 25% sodium methoxide in methanol (0.3 ml) was added. Tert-butyl hypochlorite (0.3 ml) was added to the mixture under stirring at room temperature under N₂, and the reaction was done with minimal exposure to light. After 1 hour, acetone was added to decompose excess hypochlorite. Methanol and excess acetone were distilled off under reduced pressure. Ethyl acetate (10 ml) was added to the remaining residue, followed by 1M HCl (10 ml) to dissolve the solid sodium methoxide. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 x 10 ml). The combined organic layer was dried with anhydrous Na₂SO₄ and the solvent was removed under reduced pressure to give a colourless crystalline solid. 97% yield of 7 was obtained.

2,4-dimethoxy-3-aminomethylbenzoate (3)

7 (90 mg, 0.373mmol, 1 equiv.) was dissolved in 11 ml of absolute ethanol. 24.5 mg of Adam’s catalyst (PtO₂/C) was added to the solution. The suspension was then stirred under 1 atm of H₂ gas at room temperature for 2h. The reaction mixture was filtered through Celite and the filtrate evaporated under under reduced pressure to give the crude product. The crude product was purified by silica gel column chromatography using dichloromethane as eluent. 97% yield was obtained as pale brown crystalline solid.

RESULTS AND DISCUSSION

Regiospecificity of the desired electrophilic substitution position for Friedel-Crafts acylation is greatly influenced by the substituents present on 4 as well as its plane of symmetry. Polysubstitution do not occur as the introduction of a single acyl group onto the aromatic ring deactivates and prevent further electrophilic attack.

The improved synthetic scheme showed forms the ester 7 directly from 6, without having to form the acid intermediate. By replacing the nucleophile from water to methoxide ion (or methanol), the ester 7 could be formed directly. As an anhydrous condition is required, the reagents used are modified accordingly to sodium methoxide in methanol as the base with tert-butyl hypochlorite as the chlorinating agent. This modified Lieben haloform reaction provides a simpler conversion as compared to the two-step process of forming the carboxylic acid followed by esterification.
Figure 2. Modified 1st Generation Synthetic Route via 9 and 2nd Generation Synthetic Route (with Baeyer-Villiger Oxidation as a key step) via 10.

Suggested reagents: (a) K$_2$CO$_3$, MeI, (in acetone) gentle reflux;[9] (b) AlCl$_3$, trichloroacetyl chloride, CH$_2$Cl$_2$, 0°C;[11] (c) MeONa/MeOH, dry MeOH; (d) H$_2$, PtO$_2$/C in ethanol;[10] (e) AlCl$_3$, Pivaloyl chloride, CH$_2$Cl$_2$, 0°C;[11] (f) Baeyer Villiger Oxidation; (g) H$_2$, PtO$_2$/C (in ethanol)[10]

Friedel-Crafts acylation of 8 did not yield the desired products. Firstly, it could be due to the steric bulk of the trichloroacetyl (or pivaloyl) cation which hindered the rate of the electrophilic substitution. The aromatic ring may not be sufficiently electron-rich, due to the electron-withdrawing nitro group, to compensate for this decrease in rate of electrophilic substitution and as such giving opportunity for the decarbonylation of acylium cation to occur.

CONCLUSION

Improvements made to the 1st Generation Synthetic Route (with Lieben haloform reaction as a key step) by synthesising 7 directly from 6 with tert-butyl hypochlorite in alkaline methanol gave an overall yield of 55%. This obtained overall yield over four steps could be considered comparable with those reported in the literature.[4,12] This scheme is a plausible alternative in terms of the chemistry as well as a cheaper alternative as the reagents used in our scheme are common reagents.

The synthesis of another protected form of the aromatic fragment was investigated but the procedure thus used was unable to form the desired intermediate to carry on with the synthesis. However, there is still potential in achieving the desired target with a different chemistry.

REFERENCES