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Pilot-Scale Lipase-Catalyzed Regioselective Acylation of Ribavirin in Anhydrous Media in the Synthesis of a Novel Prodrug Intermediate

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Abstract:

The ester 4 was synthesized via *Candida antarctica* lipase-catalyzed acylation of ribavirin (3) with the oxime ester of L-Cbz-Ala (2) in anhydrous THF. The reaction was highly regioselective, resulting in the exclusive acylation of the 5′-hydroxyl. The process practiced on pilot-plant scale produced 82 kg of 4 in three batches in an average isolated yield of 82%.

Introduction

Ribavirin is a powerful antiviral agent used in combination with alfa-2β interferon to treat hepatitis C.[1−5] Although this therapy is very effective in eradicating hepatitis C virus, it has several side effects associated with the highly variable pharmacological characteristics of ribavirin.[2,3,6−8] Patients with high bioavailability experience an increased risk of hemolysis and anemia, while patients with low bioavailability report a low rate of response. It was suggested that the administration of ribavirin in the form of a prodrug might improve its pharmacokinetic profile and reduce side effects. Indeed, a series of preclinical evaluations demonstrated that the bioavailability and variability of the alanine ester of ribavirin, **5** (Figure 1) are improved compared to those of ribavirin. To satisfy the prodrug requirements to be used in toxicological studies, formulation development, and early clinical trials, an efficient synthesis of **5** was required.

The route centered on the synthesis of the intermediate **4**, (to be converted into **5** via hydrogenation). The attempts to obtain **4** via chemical acylation of the unprotected ribavirin were not successful, resulting in a complex mixture of mono-, di-, and triacylated products. The alternative strategy based on protection of the two secondary hydroxyls as the acetonide was more successful, giving a lead to a commercial synthetic route. However the estimated 6-month time frame for supplying **4** via this indirect three-step process was considered to be unacceptably long. To circumvent this problem we decided to investigate the possibility of providing the first deliveries of **4** via a direct enzymatic route.

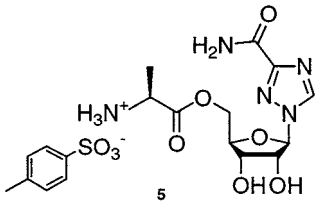

Figure 1.

The validity of the enzymatic approach was supported by several examples of the selective enzymatic acylation of the primary hydroxyls of various sugars and sugar derivatives.[9−16] For example, Novozyme-435 catalyzed acylation of the 5′-hydroxyl of purine arabinoside with vinyl acetate in THF proceeded in 99% yield.[12] The acylation of inosine and 2′-deoxyinosine by the same enzyme in pyridine and THF proceeded in 85% yield.[15,16] Moreover, the acylation of ribonucleotides with recombinant protease subtilisin gave the desired product in 65−90% yields with <5% of diacylated byproducts.[14]

Herein we report the development of an efficient pilot-scale one-pot synthesis of **4**, that was used for the production of ∼80 kg of the intermediate **4** (Scheme 1). Subsequent conversion of **4** into **5** via hydrogenation proceeded without racemization and was also successfully scaled-up. Details of the latter investigation will be reported elsewhere.

Results and Discussion

A selective acylation of the 5′-hydroxyls of several nucleosides with amino acid derivatives in the presence of

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(1) Pianko, S.; McHutchison, J. G. *J. Gastroenterol. Hepatol.* **2000**, *15*, 581−586.
(2) Sauleda, S.; Juarez, A.; Esteban, J. I.; Altisent, C.; Ruiz, I.; Puig, L.; Esteban, R.; Guardia, J. *Hepatology (Philadelphia)* **2001**, *34*, 1035−1040.
(3) Santagostino, E.; Rumi, M. G.; Rivi, M.; Colombo, M.; Mannucci, P. M. *Blood* **2002**, *99*, 1089−1091.
(4) Ferenci, P.; Stauber, R.; Steindl-Munda, P.; Gschwantler, M.; Fickert, P.; Datz, C.; Muller, C.; Hackl, F.; Rainer, W.; Watkins-Riedel, T.; Lin, W.; Krejs, G. J.; Gangl, A. *Eur. J. Gastroenterol. Hepatol.* **2001**, *13*, 699−705.
(5) Ferenci, P.; Brunner, H.; Nachbaur, K.; Datz, C.; Gschwantler, M.; Hofer, H.; Stauber, R.; Hackl, F.; Jessner, W.; Rosenbeiger, M.; Munda-Steindl, P.; Hegenbarth, K.; Gangl, A.; Vogel, W. *Hepatology (Philadelphia)* **2001**, *34*, 1006−1011.
(6) Chutaputti, A. *J. Gastroenterol. Hepatol.* **2000**, *15*, E156−E163.
(7) Ascione, A.; De Luca, M.; Di Costanzo, G. G.; Picciotto, F. P.; Lanza, A. G.; Canestrini, C.; Morisco, F.; Tuccillo, C.; Caporaso, N. *Curr. Pharm. Des.* **2002**, *8*, 977−980.
(8) Bonkovsky, H. L.; Stefancyk, D.; McNeal, K.; Banner, B. F.; Liu, Q.; Zucker, G. M.; Israel, J.; Stagias, J.; Colker, J. *Dig. Dis. Sci.* **2001**, *46*, 2051−2059.
(9) Moris, F.; Gotor, V. *Tetrahedron* **1993**, *49*, 10089−10098.
(10) Moris, F.; Gotor, V. *J. Org. Chem.* **1993**, *58*, 653−660.
(11) Ozaki, S.; Yamashita, K.; Konishi, T.; Maekawa, T.; Eshima, M.; Uemura, A.; Ling, L. *Nucleosides Nucleotides* **1995**, *14*, 401−404.
(12) Mahmoudian, M.; Eaddy, J.; Dawson, M. *Biotechnol. Appl. Biochem.* **1999**, *29*, 229−233.
(13) Singh, H. K.; Cote, G. L.; Hadfield, T. M. *Tetrahedron Lett.* **1994**, *35*, 1353−1356.
(14) Wong, C. H.; Chen, S. T.; Hennen, W. J.; Bibbs, J. A.; Wang, Y. F.; Liu, J. L. C.; Pantoliano, M. W.; Whitlow, M.; Bryan, P. N. *J. Am. Chem. Soc.* **1990**, *112*, 945−953.
(15) Prasad, A. K.; Wengel, J. *Nucleosides Nucleotides* **1996**, *15*, 1347−1359.
(16) Ciuffreda, P.; Casati, S.; Santaniello, E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1577−1582.

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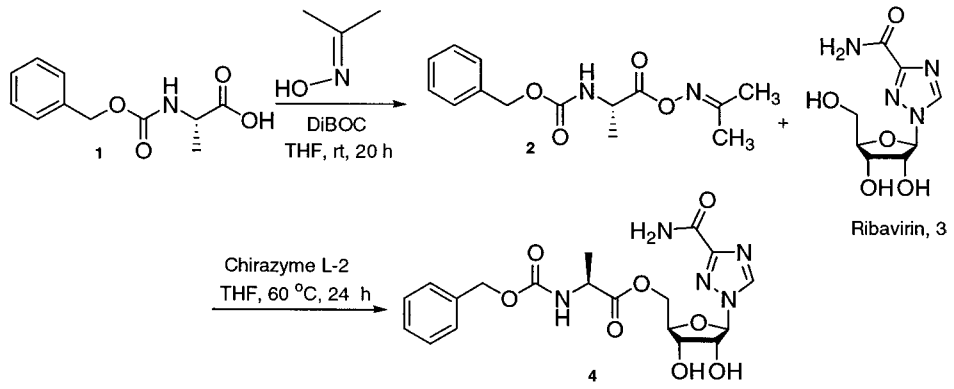
Scheme 1



Candida antarctica lipase B has been described.[17] Despite the authors' assertion that the procedure is limited to straight-chain amino acids such as glycine and β-alanine, we found that a branched amino acid, α-alanine, was also accepted by the lipase. While the degree of conversion at room temperature was indeed low (most likely due to a negligible solubility of ribavirin in THF below 30 °C), at 45 °C the acylation of ribavirin (25 g/L) with **2** in anhydrous THF in the presence of Chirazyme L-2 (20 g/L) reached ~20% within 24 h. At 60 °C the conversion improved to 40% within 24 h and continued to increase steadily, reaching 61% within 48 h. Surprisingly, the limited solubility of ribavirin in THF (even near the solvent's boiling temperature) did not prevent the reaction from proceeding virtually to completion: 90−95% conversion of ribavirin was achieved at 60 °C in the presence of 1.5 molar excess of the oxime ester.

Maintaining a low amount of moisture (<0.05%) in the solvent was found to be of critical importance for achieving high product yield. The presence of as low as 0.2% water in THF led to a significant lipase-catalyzed hydrolysis of **2** resulting in a major yield reduction. On the contrary, the moisture content of the enzyme preparation had only a marginal effect on the product yield, and therefore the standard commercial preparation of the catalyst (containing 1−2% water) was used for the production with no moisture or humidity control.

In an attempt to further optimize the product yield, the acylation of ribavirin with a highly activated trifluoroethyl ester of alanine was investigated. However, this substitution did not lead to any noticeable yield improvement and therefore **2** continued to serve as an acyl donor in all subsequent experiments. The effect of the reaction medium on the acylation of ribavirin with **2** was also examined. The product yield in acetonitrile, dioxane, *tert*-amyl alcohol, and acetone was similar to that in THF. For practical reasons that included the solvent cost, purity, low moisture content, and the ease of handling, THF remained the solvent of choice.

One-Vessel Synthesis. The coupling of **1** with acetone oxime in the presence of di-*tert*-butyl dicarbonate in THF was carried out as described previously.[18] The reaction was found to be clean and efficient giving **2** in >96% yield. The absence of byproducts prompted us to investigate the possibility of converting **1** into **4** without isolating the intermediate **2**. To that end, **1** was esterified with acetone oxime following the standard procedure (see Experimental Section). The reaction mixture was then diluted 3-fold with THF, ribavirin was added, and the acylation reaction was initiated by addition of the lipase. The immobilized catalyst remained completely insoluble in THF, forming a uniform free-flowing suspension. Due to its poor solubility in THF most of the ribavirin remained in suspension as well. Nevertheless, the reaction rate was found to be independent of the rate of mixing, indicating that the rate of conversion was not controlled by diffusion. Following a 24-h incubation at 60 °C **4** was isolated in 85% yield, comparable to that obtained in the two-step procedure (85%). For obvious reasons the sequential one-vessel procedure was selected for a scale-up.

Product Isolation. Due to its amorphous nature, high solubility in water, and hygroscopicity, the product **5** was not amenable to purification. Therefore, the isolation/purification of the last intermediate, **4**, became critical in controlling the level of impurities in the final product. Fortunately, the crystalline nature of **4** led to the development of a robust and efficient purification procedure. Following reaction completion the enzyme and unreacted ribavirin (~4%) were removed by filtration at ambient temperature (the problem of slow filtration caused by attrition of the enzyme beads was circumvented by adding Celite to the reaction mixture prior to filtration). The filtrate was then concentrated by distillation, and the product was precipitated by the addition of methyl *tert*-butyl ether (MTBE), leaving the unreacted starting materials, the oxime ester intermediate, and a small amount of the diacylated ribavirin (~0.5%) in solution. Maintaining the crystallization temperature between 40 and 50 °C was found to be crucial for producing easy-to-filter material. The residual ribavirin (<1%) was then removed by resuspending the filter cake in water at 40−50 °C.

The aforementioned procedure was transferred to the pilot plant where it was used to produce ~80 kg of **4** with >98% purity in 80−85% isolated yield. It provided sufficient amount of material to conduct the toxicological and early formulation studies. Most importantly, the enzyme-based route offered an opportunity for an earlier drug entry into

(17) Moris, F.; Gotor, V. *Tetrahedron* **1994**, *50*, 6927−6934.
(18) Pozdnev, V. F. *Int. J. Pept. Protein Res.* **1992**, *40*, 407−414.

the clinical phase than that provided by the alternative chemical approach.

Experimental Section

General. Commercial reagents and solvents of the highest purity available were used without further purification. For large-scale reactions, the water content in THF was <0.02% (determined by Karl Fisher analysis). The di-*tert*-butyldicarbonate (mp 23 °C) was stored between 30 and 35 °C for melting before using. Chirazyme L-2 enzyme (lipase B from *Candida antarctica* immobilized on a microporous acrylic resin) was purchased from Roche Molecular Biochemicals, Germany.

TLC was performed on Analtech precoated HLF-254 plates and visualized using light or applying a solution of AMC (6.25 g of ammonium molybdate, 2.5 g of cerium (IV) sulfate in 250 mL of 10% aqueous H_2SO_4) followed by heating.

Chiral HPLC was performed using a Chiralpak AS column (4.6 mm × 250 mm) (Chiral Technologies, Exton, PA) eluted with 35% EtOH/heptane mobile phase (40 °C, 0.7 mL/min; UV detection at 210 nm).

Reversed-phase HPLC was performed on a YMC ODS-AQ S5 120A column (YMC Inc, Wilmington, NC) 4.6 mm × 150 mm at 1 mL/min flow rate using the following conditions: 100% solvent A for 9.0 min, followed by a linear gradient to 100% solvent B in 16 min (A = 7.0 mM Na_2SO_4 in 0.02% aqueous H_3PO_4; B = 85% MeCN and 15% solvent A), at a flow rate of 1.0 mL/min and 25 °C; UV detection at 220 nm.

NMR spectra were recorded on a Bruker AVANCE 400 spectrometer using TMS (0.00 ppm) as an internal standard. High-resolution MS was performed on JEOL JMS-HX110A spectrometer.

***O*-(*N*-Benzyloxycarbonyl-L-alanyl)acetoxime, 2.** Cbz-Ala-OH (50 g, 0.22 mol), acetone oxime (19.64 g, 0.27 mol), and di-*tert*-butylcarbonate (53.77 g, 0.24 mol) were dissolved in THF (450 mL) and pyridine (13 mL) and incubated at 20 °C for 20 h. EtOAc (300 mL) and water (300 mL) were added to the reaction mixture, the organic layer was separated, and the aqueous layer was extracted twice with EtOAc (2 × 100 mL). The combined organic extracts were successively washed with 5% aqueous K_2CO_3, water, and brine (300 mL each), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to provide 62.81 g (100% yield) of **2** as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (d, 3H, J = 7.2 Hz), 1.95 (s, 3H), 2.01 (s, 3H), 4.50 (t, 1 H, J = 7.4), 5.08 (s, 2H), 5.5−5.48 (br, 1H), 7.30−7.33 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.3, 20.0, 23.1, 49.9, 68.1, 129.07, 129.1, 129.5, 137.2, 156.5, 166.2, 171.6

Benzyloxycarbonyl Alanine Trifluoroester (Cbz-Ala-TFA). Trifluoroethanol (3.0 g, 30 mmol), pyridine (1.4 g, 18 mmol), and di-*tert*-butyl carbonate (6 g, 27.5 mmol) were added to a solution of Cbz-Ala-OH (5.58 g, 25 mmol) in THF (50 mL). After stirring for 22 h at room temperature the reaction mixture was poured into water and extracted with EtOAc. The combined organic extracts were washed with 5% aqueous K_2CO_3, water, 5% aqueous H_2SO_4, and brine, dried over Na_2SO_4, and filtered. The solution was concentrated in vacuo to afford the benzyloxycarbonyl alanine trifluoroester as a solid in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (d, 3H, J = 6.40 Hz), 4.18−4.35 (m, 3H), 5.11 (d, 2H part of ABC system), 5.21 (br s, 1H), 7.33−7.36 (m, 5H). ^{13}C NMR (100 MHz, CDCl3) δ 19.3, 50.7, 62.0 ($^2J_{CF}$ = 36.7 Hz), 68.3, 124.3 ($^1J_{CF}$ = 124.3), 129.13, 129.3, 129.6, 137.1, 156.6, 172.6.

Carbamic Acid [2-{4-[3*S*-(Aminocarbonyl)-1H-1,2,4-triazol-1yl]-1-α-D-ribofuranosyl}-1-methyl-2-oxyethyl]-phenyl Methyl Ester, 4. One-Pot Acetylation Procedure. Cbz-Ala-OH (27.5 g, 0.12 mol) in THF (287 mL), pyridine (7.7 g, 0.1 mol), acetone oxime (9.5 g, 0.13 mol), and di-*tert*-butyl carbonate (29.6 g, 0.14 mol) were placed in a jacketed flask and stirred under nitrogen at 22 °C for 20 h. THF (550 mL), ribavirin (20 g, 0.082 mol), and lipase Chirazyme L-2 (16 g, 20 g/L) were then added, and the mixture was heated at 60 °C with stirring. After 45 h the enzyme was removed by filtration, and the solution was concentrated and dried under vacuum overnight to give a sticky oily solid. This solid was dissolved in THF (350 mL), and MTBE (600 mL) was added in portions under reflux. The product precipitated gradually as the refluxing proceeded. Following filtration 31.5 g (85.6%) of **4** was obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (d, 3H, J = 7.6 Hz), 4.13−3.38 (m, 3H), 4.36−4.29 (m, 3H), 5.01 (d, 2H, J_{ABC} = 2.0 Hz), 5.42 (d, 1H, J = 6.0 Hz), 5.70 (d, 1H, J = 5.20 Hz), 5.91 (d, 1H, J = 3.20), 7.33−7.28 (m, 5H), 7.66 (s, 1H), 7.77 (d, 1H, J = 7.60), 7.88 (s, 1H), 8.83 (s, 1H). ^{13}C (100 MHz, DMSO-d_6) δ 18.56, 51.01, 66.31, 67.09, 71.96, 75.82, 83.19, 93.04, 129.19, 129.28, 129.81, 138.33, 146.80, 146.94, 157.24, 158.95, 161.78, 174.11. FAB HRMS calcd for $C_{29}H_{24}N_5O_8$ (MH^+) m/z 450.1625, found 450.1623.

Large-Scale Synthesis of 4. Cbz-Ala-OH (24.8 kg, 111.1 mol) acetone oxime (8.5 kg, 116.3 mol), di-*tert*-butyl dicarbonate (28.3 kg, 129.7 mol), and pyridine (6.9 kg, 87.2 mol) were dissolved in THF (260 L) and stirred at 22 °C. After 24 h ribavirin (18.0 kg, 73.7 mol), Chirazyme L-2 (14.4 kg), and THF (495 L) were added, and the mixture was agitated at 55−60 °C for 24 h. Celite was added to the reactor to assist the filtration, the reaction mixture was filtered, the filtrate was clarified by recycling through a 15 μm in-line cartridge filter and concentrated to about 216 L by atmospheric pressure distillation. After cooling to 40−50 °C MTBE (250 L) was slowly added. Following precipitation, the mixture was cooled to 25 °C over 1 h, and the precipitated product was filtered. The filter-cake was dried under vacuum at 50 °C for 12−18 h. It was then resuspended in 15 vols of water and stirred at 45 °C for about 7 h to remove unreacted ribavirin. The suspension was then filtered and dried in an air-draft oven at about 45 °C to give 27.5 kg of **4** (83% yield, 99.3% purity, >99.9% de).

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