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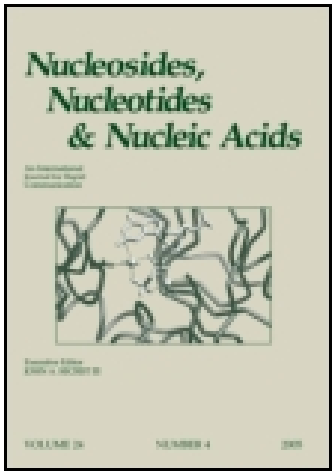

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Discovery of ANA975: An Oral Prodrug of the TLR-7 Agonist Isatoribine

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DISCOVERY OF ANA975: AN ORAL PRODRUG OF THE TLR-7 AGONIST ISATORIBINE

Alan X. Xiang, Stephen E. Webber, Bradley M. Kerr, Erik J. Rueden, Joseph R. Lennox, Gregory J. Haley, Tingmin Wang, John S. Ng, Mark R. Herbert, David L. Clark, Virginia N. Banh, Wei Li, Simon P. Fletcher, Kevin R. Steffy, Darian M. Bartkowski, Leonid I. Kirkovsky, Lisa A. Bauman, and Devron R. Averett □ *Anadys Pharmaceuticals, Inc., San Diego, California, USA*

□ *ANA975, a 5-amino-3-β-D-ribofuranosyl-3H-thiazolo[4,5-*d*]pyrimidin-2-one derivative, was synthesized in the search of an oral prodrug of isatoribine, a small molecule toll-like receptor 7 (TLR-7) agonist. Several strategies were studied to enable the kilogram-scale synthesis of ANA975. Three general total syntheses are described. In the phase I clinical study of ANA975 against hepatitis C virus (HCV), conversion to isatoribine in plasma was rapid and effective, delivering levels of isatoribine that have been shown to be clinically relevant.*

Keywords ANA975; isatoribine; prodrug; nucleoside; hepatitis C; toll-like receptor

INTRODUCTION

Isatoribine (**1**)[1] is a small molecule toll-like receptor 7 (TLR-7) agonist[2] and an activator of innate immunity. In a clinical study in patients with chronic HCV infection, IV administration of isatoribine once or twice daily for 1 wk was well tolerated and caused plasma HCV RNA concentrations to decrease in association with an increased expression of interferon response genes (Figure 1). After 1 wk of treatment with intravenous isatoribine 800 mg daily, the extent of the HCV viral load decline resembled that reported at the end of 1 wk of treatment with interferon-alpha (IFNα) based regimens, although the time course (kinetics) of viral load decline differs from that reported for IFNα based treatments.[3]

An oral product that delivers isatoribine to systemic circulation is preferred over parenteral administration. However, the oral administration of isatoribine is subject to difficulties arising from poor absorption, poor solubility, and degradation in the digestive tract. In addition, the oral

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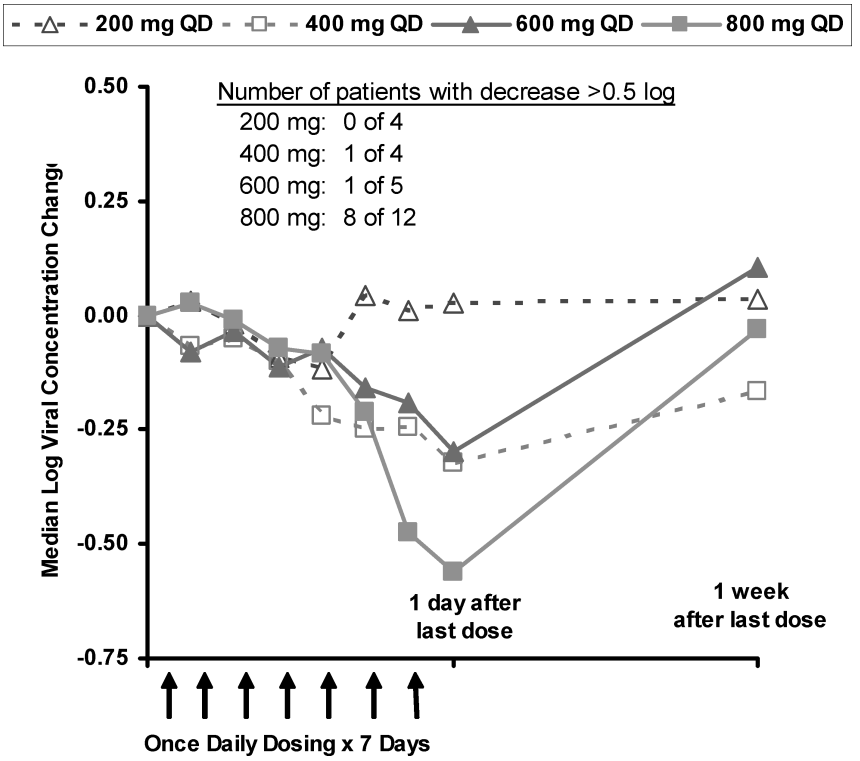

FIGURE 1 Antiviral effect of isatoribine (**1**).

administration of an unmasked immunomodulator such as isatoribine could lead to undesirable side effects in the gastrointestinal tract.[4] In the search for an oral prodrug of isatoribine, ANA975 (**6**) has emerged as the optimum candidate. In vitro testing has shown that ANA975 is converted to isatoribine via a combined mechanism of hydrolysis by esterases and oxidation by aldehyde oxidase.[5] Phase I clinical trial data of ANA975 indicates that the bioavailability of ANA975 is greater than 85% (Figure 2). In the phase I study, conversion to isatoribine in plasma was rapid and effective, achieving plasma isatoribine exposures comparable to those previously shown to induce immune biomarkers and reduce plasma HCV RNA in chronically infected patients.[6]

Here we report three total syntheses of ANA975. A detailed investigation of these routes has enabled us to produce ANA975 in kilogram quantity in a cost-effective and time-efficient manner.

SYNTHESES

Our initial strategy for synthesizing ANA975 utilized isatoribine as the building block (Scheme 1). In this synthesis, the 7-position of the nucleoside

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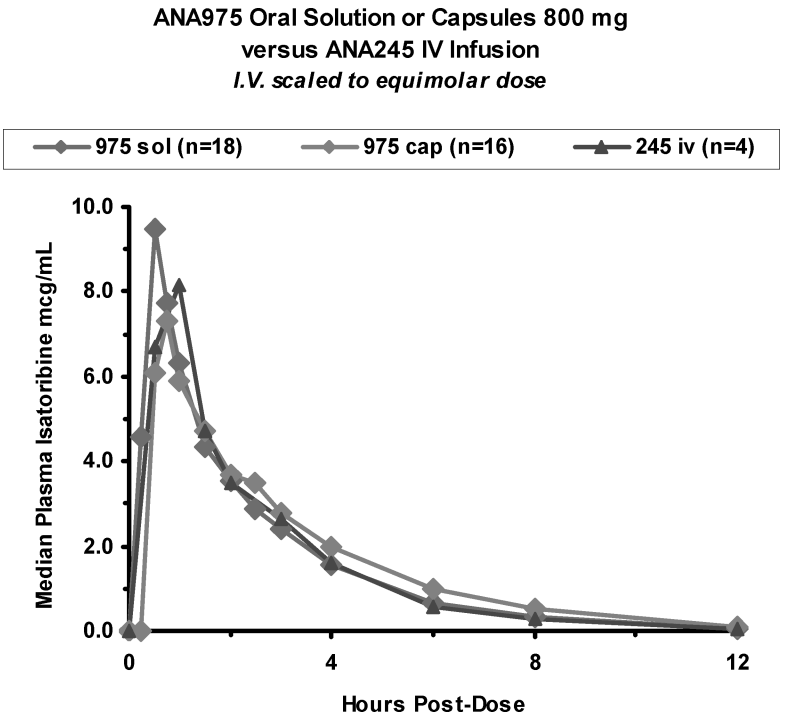
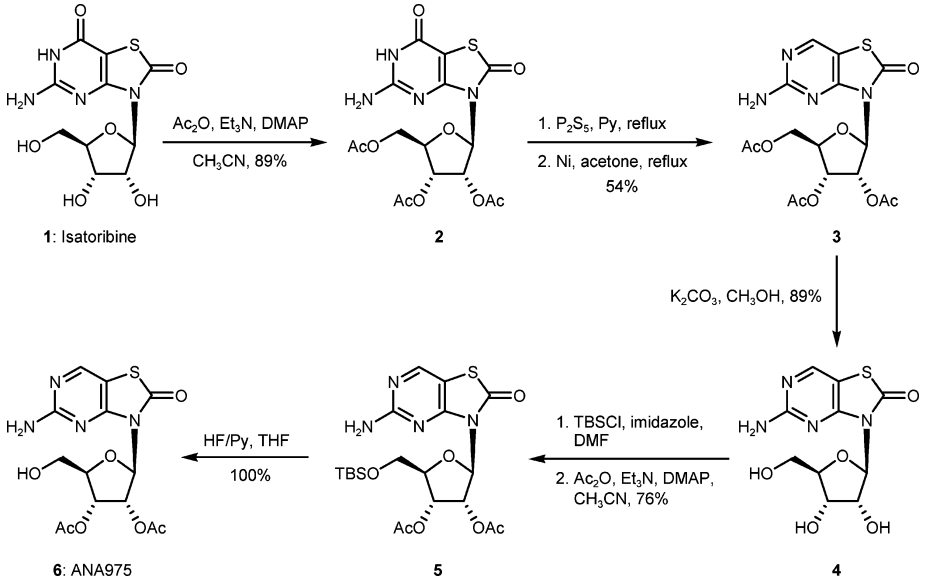


FIGURE 2 Pharmacokinetics of isatoribine following oral administration of ANA975 (**6**) oral solution and capsule to healthy volunteers compared to intravenous administration of isatoribine to HCV infected patients.



SCHEME 1 Original synthesis of ANA975 (**6**).

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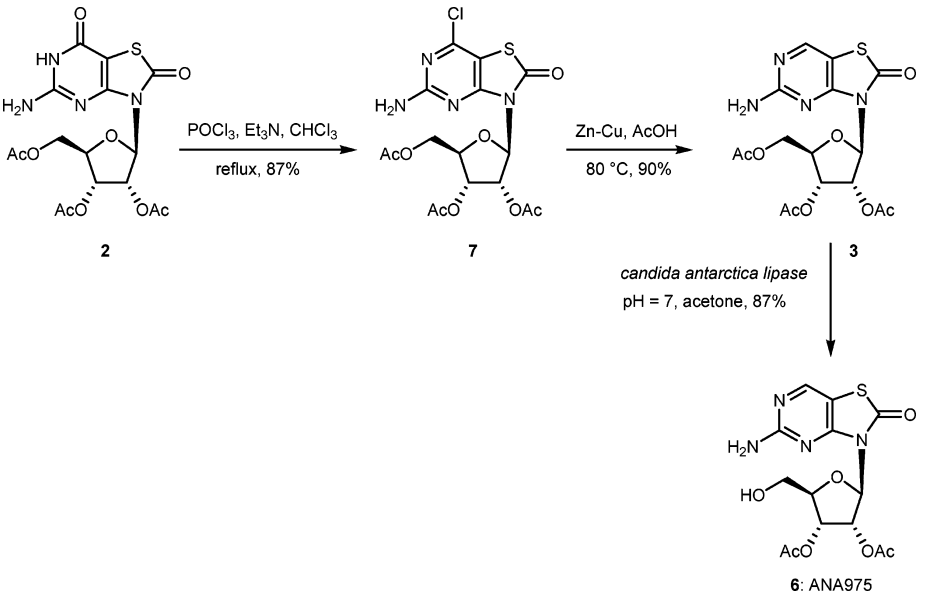

SCHEME 2 Second generation of ANA975 (**6**) synthesis.

base was modified via a key hydro-desulfurization through treatment of Raney nickel.

While the original approach provided us with an adequate quantity of ANA975 for initial testing, the route was fairly lengthy and the overall yield was low (32%). Additionally, the Raney-Ni reduction proved to be the bottleneck step due to its inflammablility and tedious workup. To avoid this problem, we investigated a hydro-dehalogenation approach for the synthesis of ANA975 (Scheme 2). The 7-chloro derivative of isotoribine **7** was reduced by a Zn-Cu couple in AcOH at 80°C to afford 7-deoxy tri-acetate **3** in 90% yield. We also utilized a selective acetate hydrolysis *via candida antarctica lipase* (CAL) to replace the labor-intensive sequence of protections and de-protections at the end of our initial synthesis.[7] Thus, we were able to synthesize ANA975, starting from isatoribine, in four steps with a combined yield of 61%. This second generation synthesis of ANA975 was later scaled up in multi-kilogram quantities.

The first and second generation syntheses of ANA975 have both utilized isatoribine as the starting material. While isatoribine is inexpensive to obtain, it does require a seven-step synthesis[1] starting from commercially available material. We therefore investigated a novel approach to construct ANA975 utilizing 5-amino-3*H*-thiazol[4,5-*d*]pyrimidin-2-one[8] (**10**) prepared in two steps from 2,4-thiazolidinedione (**8**) (Scheme 3). The heterocyclic base was coupled with tetra-acetate-D-ribose (BSA, TMSOTf, CH_3CN), followed by selective hydrolysis with CAL at neutral pH to afford ANA975 in 18% overall yield. The new route uses inexpensive commercially

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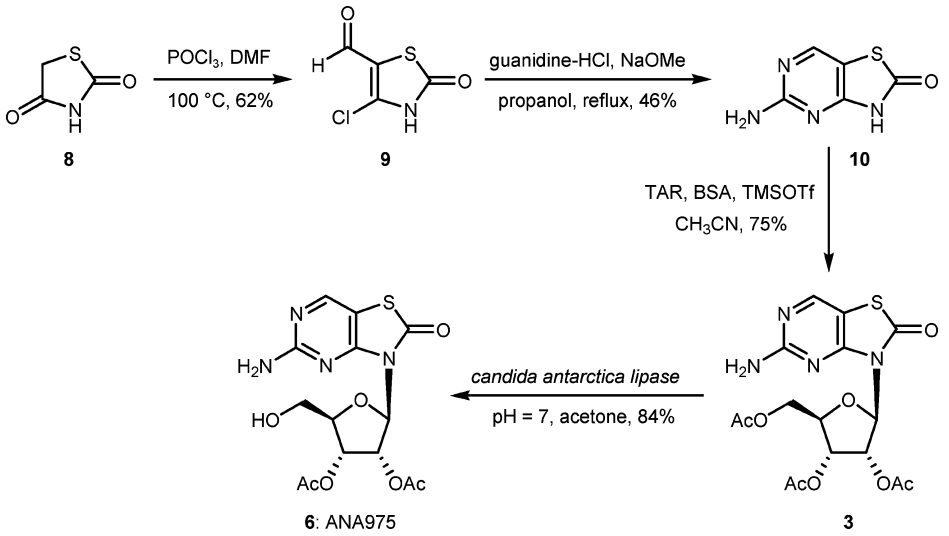

SCHEME 3 Third generation of ANA975 (**6**) synthesis.

available starting materials, requires only four synthetic steps, and avoids chromatography. It replaced our previous approaches for the large-scale production of ANA975.

CONCLUSION

Three synthetic approaches for making ANA975 were developed. While the first synthesis worked well in a laboratory setting, the second and third syntheses enabled us to produce ANA975 in multi-kilogram quantities, thus effectively fulfilling the needs for clinical trials against chronic HCV and HBV infection.

NOMENCLATURE

DMAP: 4-(Dimethylamino)pyridine
TBSCl: *tert*-Butyldimethylsilyl chloride
TAR: 1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose
BSA: *N*,*O*-Bis(trimethylsily)acetamide
TMSOTf: Trimethylsilyl trifluoromethanesulfonate

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