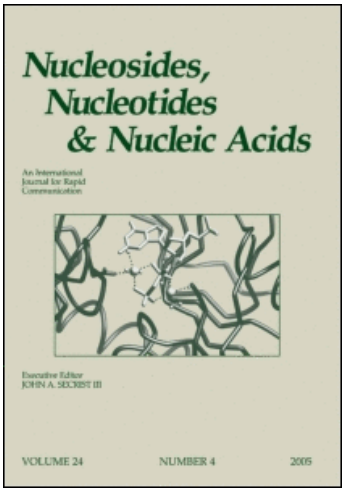

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Bruce S. Ross[a]; Quanlai Song[a]; Mingming Han[a]
[a] Isis Pharmaceuticals, Carlsbad, Califonia, USA

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KILO-SCALE SYNTHESIS PROCESS FOR 2′-*O*-(2-METHOXYETHYL)-PYRIMIDINE DERIVATIVES

Bruce S. Ross, Quanlai Song, and Mingming Han ▫ *Isis Pharmaceuticals, Carlsbad, California, USA*

▫ *We describe an improved process to produce 2′-O-(2-methoxyethyl)-pyrimidines. Starting with commercially available O-2,2′-anhydro-5-methyluridine and tris-(2-methoxyethyl)borate, we modified the ring-opening reaction conditions and changed to a continuous extraction purification method to give 2′-O-(2-methoxyethyl)-5-methyluridine. The dimethoxytritylation 5′/3′ ratios and yield were improved by the use of 2,6-lutidine as the base. Conditions to convert to the 5-methylcytidine analog and its isolation by crystallization were optimized. Final benzoylation was improved by developing a method to selectively hydrolyze benzoyl ester impurities.*

INTRODUCTION

2′-*O*-(2-Methoxyethyl)-pyrimidines are key components in several of our second generation antisense oligonucleotides in clinical development. We required a cost-efficient route which would allow for the seamless production of high purity products on a pilot plant scale.

Nucleoside alkylation methods that did not distinguish between the 2′ and 3′ hydroxyls, such as with the 2′, 3′-dibutyltin complex[1] led to mixtures that were difficult to separate. 3′,5′-Tetraisopropyldisiloxanyl protection was not stable enough for the alkylation conditions. Routes using selective alkylation on intermediates derived from glucose[2] or ribose[3] required too many steps.

We focused on the Lewis acid mediated opening on *O*-2,2′-cyclo-5-methyluridine we had discovered for 2′-*O*-methyl-pyrimidines.[4] Although the route was short, the yield was lower when using 2-methoxyethanol and the purification of the unprotected nucleoside was more challenging. Subsequent

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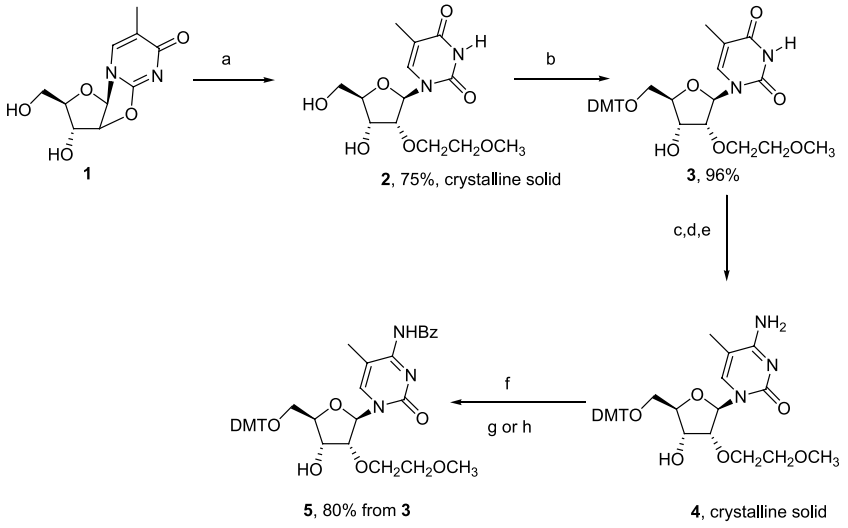
Address correspondence to Bruce S. Ross, Isis Pharmaceuticals, 2292 Faraday Ave., Carlsbad, CA 92008, USA.

protection steps required extensive chromatography and thus limited the scale and increased the cost.

RESULTS AND DISCUSSION

The starting material, *O*-2,2′-cyclo-5-methyluridine (**1**, Scheme 1), is an intermediate for the commercial production of thymidine and is available in bulk. Initially, the ring-opening reaction required a pressure reactor at 160–170°C, which limited the scale to our available pressure equipment and also increased the loss of the sugar. By increasing the concentration of a mild base such as sodium bicarbonate, the reaction temperature could be lowered to 130°C at atmospheric pressure to give 2′-*O*-(2-methoxyethyl)-5-methyl-uridine (**2**). Unlike the 2′-*O*-methyl analog, the ring opening reaction gave 10–15% of a side reaction to form dimer-like impurities such as the 5′-hydroxyl of the product reacting with the 2′ position of the starting material. This could not be removed by crystallization and required chromatography on the nucleoside or on the subsequent dimethoxytrityl derivative (**3**). This dimer could be avoided by protecting the 5′ hydroxyl of **1** as the *t*-butyldiphenylsilyl ether and opening with the 2-(methoxyethyl) borate or by protecting dimethoxytrityl as the ether and opening with 2-(methoxyethyl) aluminate. These products still required chromatography and, in the end, the easiest, scalable, and most economical solution was to purify the unprotected nucleoside **2** by continuous extraction. A number of solvent combinations were screened and we found remarkable selectivity for the desired product with dichloromethane or



SCHEME 1 (a) $B(OCH_2CH_2OCH_3)_3$(1.2 eq), $CH_3OCH_2CH_2OH$, $NaHCO_3$(8 mole%), 130°C, Argon atm, 18 h; (b) DMT-Cl (1.05 eq), −10–0°C, lutidine (2 eq), ACN; (c) TMSCl/TEA/ACN, −10–5°C. d. $POCl_3$/triazole, −20–5°C; (e) NH_4OH/dioxane (1:2), 25°C; (f) Bz_2O (1.2 eq), DMF, 25°C, 24 h; (g) 2, KOH/DMSO(1.1 eq), toluene, 25°C, 1 h; (h) KOtBu (2 eq), toluene, 48 h.

chloroform from an aqueous product solution.[5] The residue could then be crystallized efficiently from methanol/ethyl acetate to produce **2**.

Typical tritylation conditions use pyridine as a solvent and base or dimethylaminopyridine can be added. While the tritylation is selective for the 5′ hydroxyl, during the progress of the reaction we observed the formation of about a 2% 3′-*O*-DMT impurity, which then gradually decreased to below detection as the 3′,5′ bis-trityl product increased to about 10%. If any 3′-*O*-DMT impurity remained, it required a difficult chromatographic separation. We found that by using the base 2,6-lutidine[6] in a polar, aprotic solvent, the 3′-*O*-DMT impurity could not be detected and the reaction could be pushed until the bis product just began to form to maximize the yield of the product (**3**). Furthermore, we were able to reduce the excess trityl reagent after the work-up by extracting the product from an organic phase into a basic aqueous solution, acidifying with citric acid, extracting back into an organic phase and then passing the organic solution through a plug column of silica gel. After concentrating to a foam, the product met our purity requirements with a 96% yield.

We optimized the transformation to the 5-methylcytidine analog (**4**) using inexpensive, bulk 1,2,4-triazole and minimized the equivalents of phosphorus oxychloride and trimethylsilyl chloride. The unprotected product (**4**) is poorly soluble in most solvents and can be isolated by crystallization from ethyl acetate. Benzoylation with benzoic anhydride in dimethylformamide with an aqueous sodium bicarbonate wash in the work-up worked well on smaller scales, but as we increased the scale to the multi-kilogram level, new critical trace impurities were formed. The dimethoxytrityl group was removed or it migrated to the 3′ hydroxyl. We attributed this to a momentary exposure of the product to acidic water as the benzoic acid in the reaction solvent exceeded the amount of sodium bicarbonate in a saturated aqueous solution as it was mixed in. There were many solutions to this problem. First, the reaction solution could be added slowly to an excess of aqueous sodium bicarbonate with vigorous stirring. Second, the 3′ hydroxyl could be protected with a silyl before transformation to the cytidine and removed with fluoride after benzoylation. Third, the benzoic acid could be neutralized with an organic base such as triethylamine just before adding any water. This solved the first problem, but created a new problem. After the organic base addition, any excess benzoic anhydride would begin to esterify the free 3′-hydroxyl until quenched. In the case of 5-methylcytidine, we found it difficult to selectively remove the 3′-benzoate over the surprisingly labile N-benzamide. One solution is to deprotonate the amide under anhydrous conditions and thus render it inert.* On a small scale, we found that this could most readily be achieved by dissolving the product in toluene and adding slightly more than one equivalent of sodium hydride and dimethylsulfoxide. On the kilo-scale, for safety concerns, we found potassium t-butoxide would also work, although much more slowly. After remediation of most

*We wish to thank Prof. Michael Jung (UCLA) for his helpful suggestion.

impurities, the product (**5**) could be purified by passing the toluene solution through a plug column of silica gel and concentrating to a foam in 80% yield from **3**.

CONCLUSIONS

We developed a process to produce 2′-*O*-(2-methoxyethyl)-pyrimidines. Starting with commercially available *O*-2,2′-anhydro-5-methyluridine and tris-(2-methoxyethyl)borate, we improved the ring-opening reaction conditions and purification by continuous extraction to give 2′-*O*-(2-methoxyethyl)-5-methyluridine. Dimethoxytritylation 5′/3′ ratios and yield were improved by the use of lutidine as the base and isolation simplified by extractions. Conversion to the 5-methylcytidine analog and its isolation by crystallization was optimized. Final benzoylation was improved by developing a method to selectively hydrolyze benzoyl ester impurities. Taken together, these improvements have allowed us to produce these materials economically on contract with a 20 kg batch size.

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6. Tritylation process patent pending.