A Route to Lipid ALC-0315: a Key Component of a COVID-19 mRNA Vaccine

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Abstract: This paper describes a synthesis of ALC-0315 by a sequence that more than doubles the overall yield relative to the published one, and that employs much cleaner reactions, thereby facilitating purifications to a considerable extent.

Introduction

The compound known as ALC-0315, 1 (Scheme 1), is a key lipid component of the Pfizer-BioNTech COVID-19 vaccine.[1] Lipid nanoparticle (LNP) formulations of mRNA and siRNA containing 1 are also valuable research tools in the emerging field of nucleic acid therapeutics. Consequently, there is considerable interest in 1 on the part of research laboratories worldwide.

Results and Discussion

A need for research quantities of 1 and congeners prompted us to duplicate the public-domain synthesis, which is described in a patent,[2] and that involves a double reductive alkylation of 4-amino-1-butanol, 4, with aldehyde 3. The yield of 1 from 3 and 4 is reported to be of the order of 20%. It rapidly transpired that the recorded procedure is fraught with extreme difficulties. First, aldehyde 3 is obtained by PCC[3] oxidation of the corresponding alcohol 2. This step returns material contaminated with variable quantities of products of self-condensation of 3.[4] Conduct of the reductive amination step with impure aldehyde leads to the formation of myriad secondary products that complicate the purification of 1 to an unstainable degree. Aldehyde 3 thus requires careful, painstaking purification by column chromatography prior to reductive amination. Second, nonpolar 3 and highly polar 4 exhibit incompatible solubility properties. Aminoalcohol 4 is poorly soluble in solvents that readily dissolve 3 (hydrocarbons, CH₂Cl₂, CHCl₃, THF), whereas 3 (cLogP ~8) is poorly soluble in media that dissolve 4 (e.g., MeOH, MeCN, DMF). Third, the reductive amination procedure described in the patent[2] is carried out in CH₂Cl₂, in which 4 is poorly soluble, and in the presence of sodium triacetoxyborohydride, which is also poorly soluble in that solvent. The low instant solution concentrations of 4 and NaBH(OAc)₃ translate into unusually slow rates of imine formation and reduction. As a consequence, the aldehyde and/or the imine survive long enough to undergo a multitude of self-condensation reactions that generate a plethora of side products. The tedious chromatographic operations that are necessary to isolate the desired 1 lead to unacceptable losses, overall yields of 5–10%, rather than 20%, the consumption of large volumes of solvents and chromatographic supports, the generation of massive quantities of waste, and the expenditure of inordinate amounts of operator time. In our hands, the public-domain avenue to 1 wasteful, impractical, and utterly unsustainable. This induced us to research alternative routes.

The first objective of this investigation was to identify conditions for the preparation of 3 that would provide material of good quality, thus avoiding extensive chromatographic operations at the opening stages of the synthesis. The oxidation of alcohol 2 with activated DMSO[5] (Parikh-Doering,[6] Swern[7]) returned cleaner aldehyde that was more readily purified. However, an operationally simpler TEMPO-bleach oxidation[8] in a biphasic CH₂Cl₂/aqueous medium gave the best results, affording the desired 3 in 90% crude yield (Scheme 2).
material was obtained as a red oil. The origin of the color, which could not be removed by chromatography or by treatment with charcoal, remains unknown. However, the aldehyde appeared to be of very good quality\(^5\) and it was used in the reductive amination step without purification and without incident. On that note, the reductive amination of aldehydes tends to proceed best in halogenated solvents such as CH\(_2\)Cl\(_2\) that note, the reductive amination of aldehydes tends to proceed best in halogenated solvents such as CH\(_2\)Cl\(_2\) or (CH\(_3\))\(_2\)Cl\(_2\).\(^6\) Thus, a second objective was to identify a form of O-protecting that would render 4 soluble in CH\(_2\)Cl\(_2\). Hanessian’s tert-butyl-diphenylsilyl (TBDDS) group\(^{11}\) (cf. 5\(^{11,12}\)) Scheme 2 emerged as an excellent option.

Initial attempts to carry out the double reductive alkylation of 5 with 3 in CH\(_2\)Cl\(_2\) under customary conditions (stirring a CH\(_2\)Cl\(_2\) solution of 3, 5, AcOH, and NaBH(OAc)\(_3\) or NaBH\(\text{CN}\) at 0 °C to room temperature) returned complex reaction mixtures consisting of comparable amounts of desired 1, alcohol 2 resulting from reduction of the aldehyde, plus a multitude of other substances. Mass spectral and NMR data suggested that a number of such byproducts were likely to be Chichibabin-type\(^{13}\) pyridines and reduced forms thereof, signaling that the rate of reduction of iminium intermediates was too slow under the above conditions, probably because of the poor solubility of NaBH(OAc)\(_3\) or NaBH\(\text{CN}\) in CH\(_2\)Cl\(_2\) (the reaction is heterogeneous). The various iminium intermediates then become sufficiently long-lived to undergo acid-promoted (AcOH) self-condensation reactions. Furthermore, the initial concentration of aldehyde must be too high relative to the instant concentration of imine/iminium species, translating into a great extent of aldehyde must be too high relative to the instant concentration of imine/iminium species, translating into a great extent of aldehyde formation and a concomitant reduction of imine/iminium species. Thus, a second objective was to identify a form of O-protecting that would render 4 soluble in CH\(_2\)Cl\(_2\). Hanessian’s tert-butyl-diphenylsilyl (TBDDS) group\(^{11}\) (cf. 5\(^{11,12}\)) Scheme 2 emerged as an excellent option.

A solution to the first difficulty was sought in the form of a more CH\(_2\)Cl\(_2\)-soluble acyloxyborohydride.\(^{14}\) Sodium trimethyl(tri(propionyloxy))borohydride proved to be the best option.\(^{15}\) Slow addition of 3 (syringe pump) to a now homogeneous CH\(_2\)Cl\(_2\) solution of 5, AcOH, and NaBH(OOCEt)\(_3\) resulted in a much cleaner reaction. However, the extent of aldehyde reduction was still excessive, indicating that the concentration of reducing agent was now too high. Optimal results were ultimately obtained by slow (syringe pump), simultaneous addition of CH\(_2\)Cl\(_2\) solutions of aldehyde and NaBH(OOCEt)\(_3\) to a CH\(_2\)Cl\(_2\) solution of 5 and AcOH, whereupon 6 reproducibly emerged in 47–50% yield after chromatography (Scheme 3).

The synthesis was completed by desilylation of 6 with HF-pyridine\(^{16}\) to give 1 in 82% yield after chromatography. Lipid 1 is thus available in ca. 37% overall yield from 4, the costliest starting material, through a linear sequence that encompasses 3 steps.

Parallel studies on the biological activity of LNP-RNA formulation containing analogues of 1 revealed the desirability of a synthetic intermediate such as 11 (Scheme 4), which could be easily advanced to congeners of ALC-0315 exhibiting alternative acyl portions. Compound 11 was prepared by reductive alkylation of 5 with the known\(^{17}\) 6-acetoxyhexanal, 9. Thus, mono-acetylation of economical 1,6-hexanediol, 7, afforded a product mixture consisting largely of monoacetyl derivative 8, plus diacetylated product and unreacted 7. An aqueous wash of the reaction solution removed water-soluble 7. Concentration left an oily residue consisting of an approximately 84:16 (\(^1\)H NMR) mixture of 8 and the corresponding diacetate. The isolation of the desired 8 was greatly facilitated by its mediocre solubility in hexanes. Thus, washing the residue with hexanes removed most of the diacetyl product with marginal loss of 8, and left behind material containing no more than 3% (\(^1\)H, \(^13\)C NMR) of diacetate. Rough filtration through
silica gel removed the last of the diacetate, and subsequent Kugelrohr distillation (75 °C) afforded pure 8 (76%) a colorless oil. Oxidation by the TEMPO-bleach method16 produced 9 in 91% yield after Kugelrohr distillation. The product, a pale yellow oil, had excellent shelf life when stored at −20 °C. Multigram batches of 9 were thus prepared without difficulty and without chromatography. Reductive deacetylation of 5 with 9 under the conditions described earlier consistently furnished 10 in 77–80% yield after chromatography. Ensuing deacetylation gave diol 11 (quantitative), which can be advanced to ALC-0315 and congeners by esterification followed by desilylation. This is exemplified in Scheme 4 by the conversion of 11 by esterification with 2-hexyldecanoic acid in the presence of EDCI and congeners by esterification followed by desilylation. This is exemplified in Scheme 4 by the conversion of...