J. Chem. Chem. Eng. 8 (2014) 865-869
doi: 10.17265/1934-7375/2014.09.004

Developing an Efficient Technology for Key Intermediates Production Used in Enzymatic Synthesis of Cladribine and Nelarabine

Shetnev Anton Andreevich, Baykov Sergey Valentinovich, Smirnov Alexey Vladimirovich, Dorogov Mikhail Vladimirovich and Novozhilov Yury Vladimirovich*

Organic and Inorganic Chemistry Department, Ushinsky Yaroslavl State Pedagogical University, Yaroslavl 150000, Russia

Abstract: The pilot-scale process for preparing 2-amino-6-chloro-9-(2',3',5'-tri-O-acetyl-D-β-ribofuranosyl)-purine and 2-chloroadenosine has been developed with a total yield of the desired compounds 73% and 44.5%, respectively. These compounds are useful intermediates for enzymatic synthesis of active pharmaceutical ingredients Nelarabine and Cladribine. The starting material—commercially avaliable guanosine—was acetylated with acetic anhydride, yielding 2,3,5-tri-O-acetylguanosine, which was further deoxyhalogenated with phosphorous oxychloride in presence of tetraethylammonia chloride. Diazotization of the resulting intermediate with tert-butylnitrite leads to the corresponding ribofuranosyl-substituted 2,6-dichloropurine, which was converted to 6-chloroadenosine by reaction with methanolic ammonia.

Key words: Nelarabine, Cladribine, pharmaceutical intermediate, pilot-scale process.

1. Introduction

Cladribine and Nelarabine are highly demanded modern anti-tumor drugs, which are part of "Vital and Essential Drugs List" in Russian Federation and, as such, are important objects for pharmaceutical industry.

Scaling up known laboratory protocols for preparation of the chemicals is essential part for establishing reliable industrial technology.

The present papers are devoted to pilot-scale preparation of 2-chloroadenosine (Scheme 1a) and its regioisomer 2-amino-6-chloro-9-(D-β-ribofuranosyl)-purine (Scheme 1b), which are synthetic precursors of the abovementioned active pharmaceutical ingredients [1-3].

Recent medical studies reaffirmed high importance of these substances for pharmaceutical industry. It was shown that 2-chloroadenosine can act as cardiac protecting agent [4], adenosine receptor agonist [5] and anti-tumor drug in *β*-lymphocite leukemia therapy [6]. Its regioisomer (Scheme 1b) showed antiviral [7], antibacterial [8] activities and adenosine receptors affinity [9]. It is clear that development of the industrial technology for preparation of such substances is an important objective for developing economy.

2. Experiments

The reactions were monitored using TLC (thin layer chromatography) method (Silufol UV-254 plates were used). ^{1}H NMR (nuclear magnetic resonance) spectra were recorded with Bruker DRX (500.13 MHz) using dimethylsulfoxide d6 as solvent and tetramethylsilane as internal standard. HPLC (high-performance liquid chromatography) analysis was performed using Shimadzu LC 20 Promenence apparatus and Waters XBridge C18, 50 mm × 4.6 mm column. Mass-spectra were recorded using Agilent Technologies 6890N/5975B. Melting points were measured on Mel-Temp 3.0.

*Corresponding author: Novozhilov Yury Vladimirovich, Ph.D., Research Associate, research field: heterocycles chemistry. E-mail: unovozhilov@gmail.com.

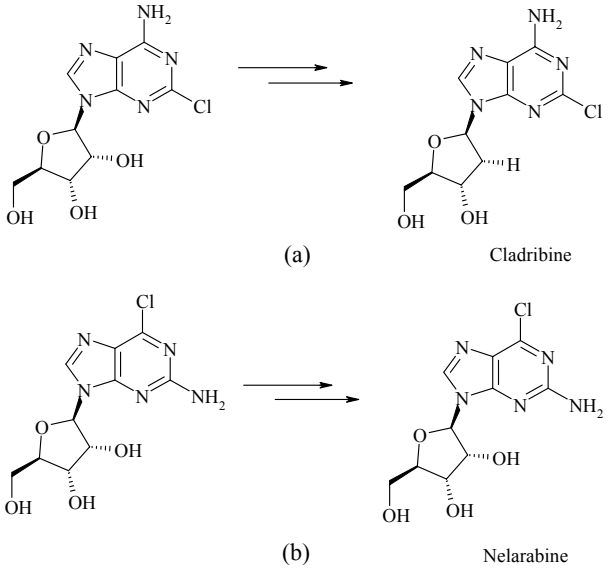
2.1 Preparation of 2,3,5-Tri-O-Acetyl Guanosine

Into enamel coated reactor 7 L of pyridine and 4 L of dimethylformamide were charged. To this solution 2.33 kg (8.15 mol) of guanosine was added. Reaction mass was heated to 50-55 °C and the mixture comprising of 3.25 kg (31.86 mol) of acetic anhydride and 3 L of dimethylformamide were dropwise added thereto. Solution was heated further to 58-60 °C and stirred for 14 h at the said temperature. Temperature was adjusted to 75-78 °C and further stirred for 2 h, after which the reaction mass was filtered through cartridge filter into the crystallization vessel. Isopropyl alcohol (14 L) was added and the solution was refluxed for 2 h. The reactor contents were chilled to 0 °C and stirred for 6 h. The resulting suspension was centrifuged and washed twice with 7 L of isopropyl alcohol. The solid was dried in vacuum at 80 °C. The crude produced was recrystallized from 26 L of isopropyl alcohol yielding 3.05 kg of title compound—2,3,5-tri-*O*-acetyl guanosine (Scheme 2 (2)).

Purity 98%. (HPLC, *Rt* (retention time) = 10.02 min). M.p. (melting point) 230-232 °C. Mass-to-charge ratio (*M*/*z*): found 410.1335 [M + H] + (calc. 410.1306).

2.2 Preparation of 2-Amino-6-Chloro-9-(2',3',5'-Tri-O-Acetyl-D-β-Ribofuranosyl)-Purine

Into 30 L rotary evaporator vessel 2,3,5-tri-O-acetylguanosine (3.00 kg, 7.30 mol) and tetramethylammonium hydrochloride (1.45 kg, 13 mol) were charged. Vacuum was applied to the vessel. To the reagents mixture 14 L of anhydrous acetonitrile was charged. The formed suspension was heated to 85 °C and refluxed under stirring. To the resulting clear solution dimethylamine (1.10 L, 8.7 mol) and freshly distilled phosphorous oxychloride (4.10 L, 44 mol) were sequentially charged and the mixture was refluxed thereafter for 20 min. The volatile components were distilled off from the reaction mixture first at atmospheric pressure and then at



Scheme 1 Precursors of Nelarabine and Cladribine.

200-30 mm Hg for 20 min. Chloroform (11 L) and water (4 L) were added to the residue and stirred until complete dissolution of solids. After cooling the reaction mass was transferred into separatory vessel and stirred for 1 h. Water phase was re-extracted with 5 L of chloroform. The combined organic layer was sequentially washed two times with 4 L of water and two times with 4 L of 5% aqueous $NaHCO_3$. The chloroform solution was dried over 500 g of anhydrous sodium sulphate, filtered and evaporated. The residue was refluxed with 45 L of isopropyl alcohol, cooled to 20 °C and further stirred for 6 h. The solid was filtered, washed with 2 L of isopropyl alcohol and dried at 60 °C in vacuum. 2.64 kg (84%) of the crude title compound was recovered. The material was purified by flash-column chromatography (1.2 kg silica-gel, 1.3 L of chloroform). The column is washed with 20 L chloroform. The eluate was concentrated and the residue was recrystallized from 16 L of isopropyl alcohol. 2.5 kg (81.2% yield) of the title product was recovered.

Purity 99%. (HPLC, Nova-Pak C18, 4.6 × 150 mm, 4 μm, gradient 0%-100% B for 20 min, *Rt* = 10.69 min). M.p. 153-154 °C. *M*/*z*: 428.1009 [M + H] + (calc. 428.0968), 430.0952 [M + H] + Cl35/37.

2.3 Preparation of 2,6-Dichloro-9-(2',3',5'-Tri-O-Acetyl-β-D-Ribofuranosyl) Purine

Into an enamel-coated vessel 1 kg (2.34 mol) of 3.550 g (4.77 mol) of pyridine chlorohydrate (dried in vacuo at 50 °C) and 20 L of methylene chloride were charged. The mixture was stirred and refluxed until complete dissolution (1 h). The reaction mass was cooled to 0 °C and 0.25 L (2.735 mol) of phosphorous oxychloride was charged thereto. The mixture was cooled further to -20 °C and 0.44 L (3.83 mol) tert-butylnitrite in 0.5 L of methylene chloride was added in drops within 150 min. After addition was complete, the mixture was stirred at -10 °C for 1 h, and further warmed to 20 °C. The reaction was quenched by adding 3 kg of ice flakes and stirring was continued for 1 h. The organic layer was separated and sequentially washed with water (2 × 3 L), 5% $NaHCO_3$ water solution (2 × 3 L), water again (2 × 3 L) and dried over 1 kg of Na_2SO_4. The dried solution was chromatographed on 4 kg of silica-gel. The column was washed with 20 L of chloroform and concentrated. After concentrating the eluate to 5 L, isopropyl alcohol (0.4 L) was added to it and distillation was continued, whereas the product began crystallizing. The suspension was maintained at 0 °C for 14 h under stirring and filtered yielding 475 g of the crude title product. The column was washed again with 20 L of chloroform-methanol mixture (20:1) and eluate was processed in the same way as described above. The crude product was recrystallized from 12 L of isopropyl alcohol yielding 749 g (71.7%) of 2,6-dichloro-9-(2',3',5'-tri-o-acetyl-β-d-ribofuranosyl) purine (Scheme 2 (4)).

Purity 95%. (HPLC, Nova-Pak C18, 4.6 × 150 mm, 4 μm, gradient 0-100% B for 20 min, *Rt* = 12.59 min). M.p. 167-169 °C. *M*/z: 447.0432 [M + H] + (calc. 447.0469), 449.0396 [M + H] + Cl35/37.

2.4 Preparation of 2-Chloroadenosine

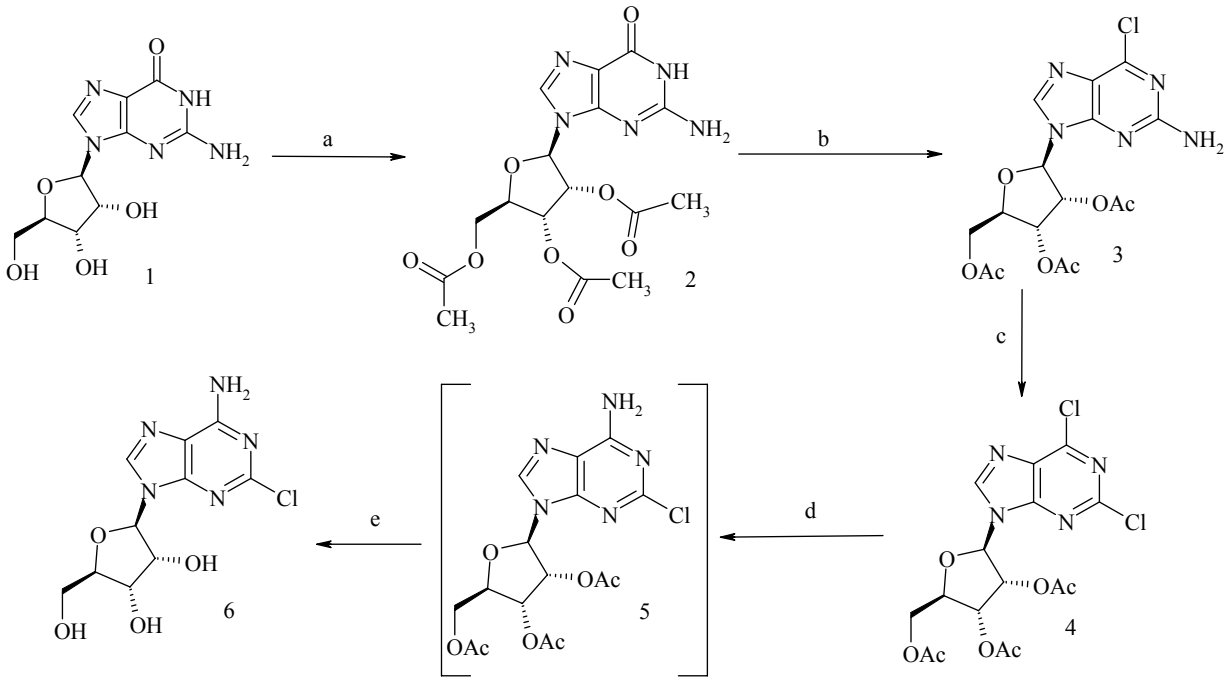
In a steel vessel 36 L of anhydrous ethylacetate, 0.423 kg (22.27 mol) of ammonium chloride and 1.089 kg (20.043 mol) of sodium methoxide were combined and stirred at 20 °C for 6 h. Upon completion, the reaction mass was cooled to 0 °C and to the formed suspension 2,6-dichloro-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl) -purine (0.9 kg) was added. The reactor contents were additionally stirred for 3 days at 0 °C and 6 h at 40 °C. The mixture was cooled to 20 °C and the crystalline solid formed was separated by filtration. The product was dissolved in 20 L chloroform-methanol (4:1) mixture and chromatographed on 1.2 kg of silica-gel. The resulting eluate was concentrated yielding 0.230 kg (85%) of 2-chloroadenosine (Scheme 2 (6)).

Purity 99.11%. (HPLC, Pak C18, 4.6 × 150 mm, 4 μm, gradient 0-30% B for 20 min, *Rt* = 11.02 min). M.p. 188-189 °C. λ_{max}, nm (ε, $M^{-1}\cdot cm^{-1}$): 263.8 (13,800). *M*/*z*: 302.0658 [M + H] + (calc. 302.0651). 1H NMR (DMSO-d6) (δ, ppm): 3.58 (m, 1H, H5'a), 3.68 (m, 1H, H5'b), 3.97 (m, 1H, H4'), 4.17 (m, 1H, H3'), 4.55 (m, 1H, H2'), 5.11 (t, 1H, OH-5'), 5.23 and 5.50 (2 d, 2H, OH-3' and OH-2'), 5.85 (d, 1H, H1'), 7.85 (m, 2H, NH_2), 8.42 (s, 1H, H-8).

3. Results and Discussion

The present protocol for preparation of (Scheme 2 (1)) and (Scheme 2 (2)) substances is based on four-step 2-chloroadenosine synthesis starting from commercially available guanosine, developed at Institute for Biological Chemistry of Russian Academy of Science.

Earlier, this method was applied for 0.001-1 mol scale synthesis. It has been shown that it can be used for up to 30 mol scale preparation after slight improvement. According to patent [10], guanosine must be acetylated by acetic anhydride in order to evade ribofuranosyl fragment epimerisation. The acetylated guanosine is reacted further with $POCl_3$ yielding dehydroxylated product. Tetraalkylammonium chlorides, used in the process as a source of chloride ion, are highly soluble in aprotic



a: Ac_2O, Py, DMFA, 58-60 °C, 14 h; 75-78 °C, 2 h, 89.9%;

b: $POCl_3$, $Me_2NC_6H_5$, Me_4NCl, CH_3CN, 100 °C, 30 min, 79.8%;

c: $C_6H_5N{\cdot}HCl$, $POCl_3$, t-BuONO, CH_2Cl_2, -20-(-15) °C, 2 h, 71.7%;

d: EtOAc, NH_4Cl, CH_3ONa, 0-5 °C, 3 days;

e: 40 °C, 6 h, 96.3%.

Scheme 2 Synthesis of 2-chloroadenosine.

solvents, allowing rapid (15-30 min) hydroxyl group substitution thus avoiding guanosine base destruction. The intermediate 3 can be further used for 2-chloroadenosine preparation. It can also act as precursor for Nelarabine preparation after being converted to 2-amino-6-methoxy-9-(*β*-D-ribofuranosyl)-9H-purine intermediate. The third step in 2-chloroadenosine synthesis is a diazotization of Scheme 2 (3) with tert-butylnitrite and subsequent substitution of diazo group with chloride ion sourced from pyridine hydrochloride. Patent [11] suggests using liquid ammonia for 6-chlorine substitution, however, handling it requires special measures and equipment. For pilot-scale preparation, authors have successfully used NH_4Cl-NaOMe combination, which is far less hazardous to handle in contrast to liquid ammonia. HPLC analysis shows that reaction completion usually takes 68-72 h. Full conversion of Scheme 2 (3) and 2 (4) is achieved within said timeframe. Deacetylitaion is effected by heating the reaction media for 6 h at 40 °C. Total yield of the desired 2-chloroadenosine was 44.5% after four steps which is consistent with reported data [10, 11].

4. Conclusions

As a result of our efforts, we have adapted previously reported laboratory protocol for preparation of 2-chloroadenosine to pilot-scale synthesis. Purity of the product is sufficient to use it as a starting material in Cladribine enzymatic preparation. We have also shown that the present method can be successfully used to produce 2-amino-6-chloro-9-(2',3',5'-tri-O-acetyl-D-β-ribofuranosyl)-purine (Scheme 2 (3)) which is a useful intermediate for anti-tumor drug—Nelarabine.

Acknowledgments

This study was supported by Russian Federation Government Decree No. 218 of April 9, 2010 (contract No. 02.G25.31.0019, code 2012-218-03-258) for the project "Establishing production of import-replacing active pharmaceutical ingredients based on innovative technologies, enzymatic synthesis and biocatalysis" conducted by CJSC (Closed Joint Stock Company) "R-Pharm" and The Ushinsky Yaroslavl State Pedagogical University.

References

[1] Taran, S. A.; Verevkina, K. N.; Feofanov, S. A.; Miroshnikov, A. I. Enzymatic Transglycosylation of Natural and Modified Nucleosides by Immobilized Thermostable Nucleoside Phosphorylases from *Geobacillus stearothermophilus*. *Rus. J. Bio. Chem*. **2009,** *35(6),* 739-745.

[2] Du, J.; Sofia, M. J. Compounds. U.S. Patent 0,142,626, July 6, 2012.

[3] Bookser, B. C.; Raffaele, N. B. High-Throughput Five Minute Microwave Accelerated Glycosylation Approach to the Synthesis of Nucleoside Libraries. *J. Org. Chem.* **2007,** *72(1),* 173-179.

[4] Dobson, G. P. Organ Arrest, Protection and Preservation. U.S. Patent 20,050,171,050, Apr. 8, 2005.

[5] Dobson, G. P. Organ Preconditioning, Arrest, Protection, Preservation and Recovery. U.S. Patent 2,014,011,745, Aug. 7, 2004.

[6] Laurent, B.; Caroline, S.; Sabine, C.; Pascale, S.; Neste, V. D.; Bontemps, E. Mechanisms of Cell Death Induced by 2-Chloroadenosine in Leukemic *β*-Cells. *Biochemical Pharmacology* **2008,** *75(7),* 1451-1460.

[7] Masayuki, I. M. S.; Shigeru, M.; Shuetsu, F.; Tetsuya, M.; Ichiro, K.; Tokumi, M. Synthesis and Biological Evaluation of Nucleoside Analogues Having 6-Chloropurine as Anti-SARS-CoV Agents. *Bioorganic and Medicinal Chemistry Letters* **2007,** *17,* 2470-2473.

[8] Alvarez, Z.; Lee, K.; Abel-Santos, E. Testing Nucleoside Analogues as Inhibitors of Bacillus Anthracis Spore Germination *In Vitro* and in Macrophage Cell Culture. *Antimicrobial Agents and Chemotherapy* **2010,** *54(12*), 5329-5336.

[9] Volpini, R.; Marucci, G.; Buccioni, M.; DalBen, D.; Lambertucci, C. Evidence for the Existence of a Specific G Protein-Coupled Receptor Activated by Guanosine. *Chem.Med.Chem*. **2011,** *6(6),* 1074-1080.

[10] Beresin, V. B.; Dorofeeva, E. V.; Leonov, V. N. Preparation of 2,6-Dichloro-9-(2',3',5'-Tri-O-Acetyl-β-D-Ribofuranosyl) Purine. RU Patent 2,007,104,123, May 2, 2007.

[11] Beresin, V. B.; Dorofeeva, E. V.; Leonov, V. N. Preparation of 2-Chloroadenosine. RU Patent 2,007,104,121, May 2, 2007.