Short communication

# Preparative high-performance liquid chromatographic separation of fluorodeoxy sugars ☆

Mary F. Evangelisto*, Richard E. Adams, William V. Murray, Gary W. Caldwell

*The R.W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, NJ 08869-0602, USA*

## Abstract

Normal- and reversed-phase preparative chromatography methods were developed to isolate gram quantities of analytically pure 6-amino-2-chloro-9-(2-deoxy-2-fluoro-$\beta$-D-arabinofuranosyl)-9H-purine (arafluoro-2-CdA; RWJ 29727) and its $\alpha$-anomer (RWJ 48667). The complex reaction mixture (ca. 171 g), from a Parr Bomb synthesis, was prepurified by normal-phase chromatography to yield ca. 40 g. Twelve reversed-phase preparative isolations were run on a custom-packed YMC column to yield ca. 12 g of arafluoro-2-CdA (99.7%) and ca. 3 g of the $\alpha$-anomer (99.2%).

## 1. Introduction

Replacement of hydroxyl groups or hydrogen atoms with fluorine atoms provides useful derivatives for blocking or retarding metabolic processes [1–3]. Unfortunately synthesis of these compounds requires vigorous conditions such as the use of hydrofluoric acid at high temperatures [4] which results in complex mixtures. Recently 6-amino-2-chloro-9-(2-deoxy-$\beta$-D-arabinofuranosyl)-9H-purine [2-CdA (Cladribine); RWJ 26251; 1] was approved for the treatment of hairy cell leukemia (Fig. 1). In the early development of 2-CdA, it became of interest to study the pharmacological profile of 6-amino-2-chloro-9-(2-deoxy-2-fluoro-$\beta$-D-arabinofuranosyl)-9H-purine (arafluoro-2-CdA; RWJ 29727; 2). Normal- and reversed-phase preparative chromatography methods were developed to isolate gram quantities of analytically pure arafluoro-2-CdA and its $\alpha$-anomer (RWJ 48667; 3).

## 2. Experimental

### 2.1. Synthesis and HPLC chemicals

Arafluoro-2-CdA was synthesized by the following procedure. 2-O-(Imidazolylsulfonyl)-1,3,5-tri-O-benzoyl-$\alpha$-D-ribofuranose (Pfanstiehl, Waukegan, IL, USA) was treated with 48% hydrofluoric acid in a Monel Parr Bomb at 170°C to yield 2-deoxy-2-fluoro-1,3,5-tri-O-benzoyl-$\alpha$-D-arabinofuranose. This fluoro sugar was
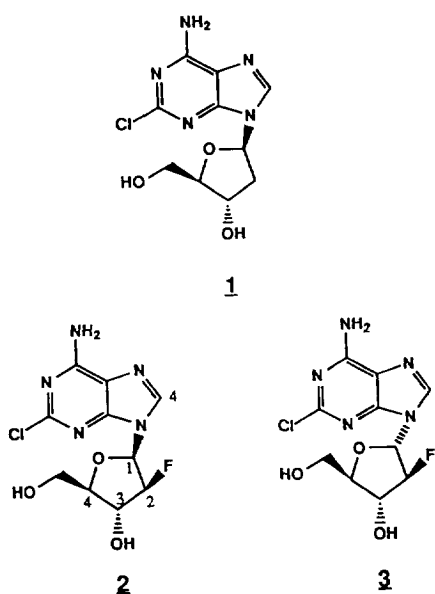
Fig. 1. Structures of 2-CdA (RWJ 26251, 1), arafluoro-2-CdA (RWJ 29727, 2) and the α-anomer of 2-CdA (RWJ 48667, 3).

treated in $CH_2Cl_2$ with 30% HBr in acetic acid dropwise over 20 min and stirred at room temperature for 16 h. The reaction mixture was cooled and adjusted to pH 8 with saturated $NaHCO_3$ solution. The organic phase was separated, washed with water, brine and dried with $Na_2SO_4$ to give 2-deoxy-2-fluoro-3,5-di-O-benzoyl-α-D-arabinofuranosyl bromide. 2,6-Dichloropurine was dissolved in $CH_2Cl_2$ and treated with 60% NaH under a nitrogen atmosphere at room temperature. Dimethylformamide was added and after 15 min the previously prepared bromide compound was added and allowed to stir for 48 h. The reaction mixture was filtered through a bed of Celite and concentrated to give a crude oil. This oil was suspended in $CH_3OH$ and treated with concentrated $NH_4OH$ in a Parr Bomb at 80°C for 16 h. The resulting reaction produced a highly impure crude oil containing 2 and 3. Reactants were obtained from Aldrich, Milwaukee, WI, USA.

HPLC-grade water, $CH_2Cl_2$, $CH_3OH$ and $CH_3CN$ were obtained from Fisher Scientific (Springfield, NJ, USA).

## 2.2. Chromatography

A complex mixture (171 g) containing 2 was prepurified via normal-phase chromatography. The sample was dissolved in 1 l of $CH_2Cl_2$–$CH_3OH$ (95:5) with heat and stirring. The sample was divided into three parts and each aliquot was separated on a Waters Prep 500A (Milford, MA, USA) using two new Waters PrepPak-500 silica gel 60 cartridge columns equilibrated with $CH_2Cl_2$–$CH_3OH$ (95:5). All fractions were checked by TLC (Merck silica gel 60 $F_{254}$, 10 × 5 cm, precoated plates, layer thickness 250 μm, Gibbstown, NJ, USA) in $CH_2Cl_2$–$CH_3OH$ (90:10). Fractions analyzing as a single spot on the TLC plates were combined and concentrated in vacuo. Subsequent analytical reversed-phase HPLC analysis of the sample showing one spot by TLC was carried out on a YMC ODS column (Wilmington, NC, USA) (250 × 4.6 mm, s-15, 120 Å, $C_{18}$) with UV detection at 254 nm using an isocratic mobile phase of $CH_3CN$–water (10:90) at a flow-rate of 1.0 ml/min (Fig. 2). The material was determined to be a mixture of 52.2% 2 and 43.0% 3 rather than a single component as indicated by TLC. The normal-phase prepurification chromatography yielded approximately 40 g of material to be further purified by reversed-phase chromatography.

Reversed-phase preparative chromatography was performed using a Waters Prep 500A instrument connected to a 500 × 50 mm stainless-steel column custom packed with 500 g of YMC ODS (s-15/30, spherical, 120 Å, $C_{18}$). The flow-rate was maintained at 50 ml/min. Samples, ranging from 3 to 5 g, were dissolved in $CH_3CN$–water (10:90), warmed, sonicated and filtered. The sample was then loaded onto the YMC column equilibrated with $CH_3CN$–water (10:90). Fraction collection was monitored by a Waters Lambda Max Model 481 LC spectrophotometer at 254 nm. A step gradient to $CH_3CN$–water (50:50) was utilized to collect fractions determined to be 99.7% pure arafluoro-2-CdA and 99.2% pure α-anomer (Figs. 3 and 4).

Analytical HPLC was recorded using a Waters 486 tunable absorbance detector, and a Waters 740 data module. A Beckman 110 solvent-deliv-
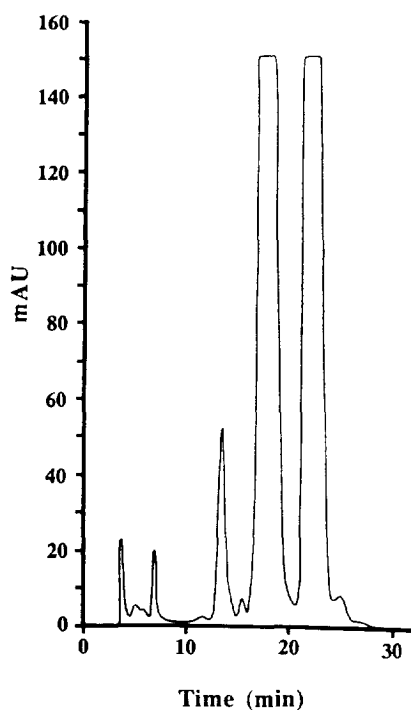
Fig. 2. HPLC of mixture of arafluoro-2-CdA (retention time 18.9 min, area percent 52.2) and the $\alpha$-anomer (retention time 23.0 min, area percent 43.0) after normal-phase prepurification. Other peaks were not identified.



Fig. 3. HPLC of arafluoro-2-CdA (retention time 18.5 min, area percent 99.7) after reversed-phase purification.

ery module (Fullerton, CA, USA) was employed as a pump.

### 2.3. Spectroscopy

Chemical ionization (DCI-CH$_4$) mass spectra were obtained on a Finnigan Incos 50 mass spectrometer (San Jose, CA, USA).

The $^1$H NMR experiments were performed on a Bruker AM-400 (400.13 MHz) Fourier transform spectrometer (Villerica, MA, USA) equipped with a 5-mm inverse broad band probe. The samples were dissolved in [$^2$H$_6$]dimethyl sulfoxide (DMSO) at ca. 10 m$M$ in 5-mm tubes at 298 K. The chemical shifts were referenced to internal tetramethylsilane (TMS). Nuclear Overhauser Enhancement (NOE) difference experiments were performed and processed using standard Bruker software. Typical
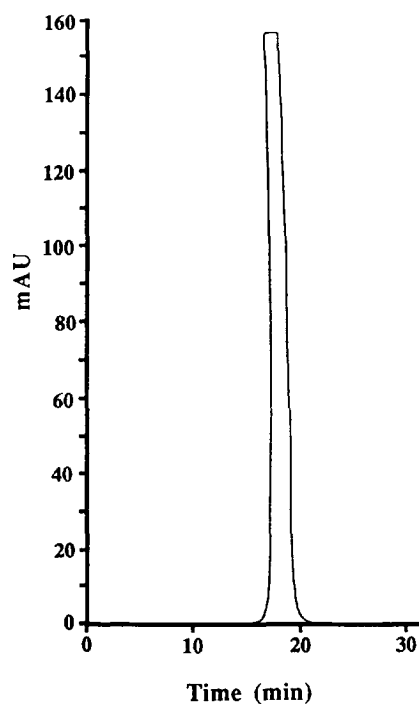
operating conditions have been previously reported [5].

### 3. Results and discussion

The reversed-phase chromatogram of the reaction mixture is shown in Fig. 2. It is noted that the majority of the impurities were removed by the normal-phase chromatography prepurification step. Reversed-phase preparative isolation of each component was accomplished and the results are shown in Figs. 3 and 4. The two major peaks at 18.9 and 23.0 min were identified as 2 and 3, respectively. Twelve reversed-phase preparative isolations were necessary to produce ca. 12 g of 2 (99.7%) and ca. 3 g of 3 (99.2%).

Both compounds were fully characterized by mass spectral and NMR analysis. The mass spectra of 2 and 3 were almost identical with each producing an $[M + H]^+$, $[M + C_2H_5]^+$, $[M + C_3H_5]^+$, $[MH - Cl]^+$, $[MH - C_4H_7O_2F]^+$,
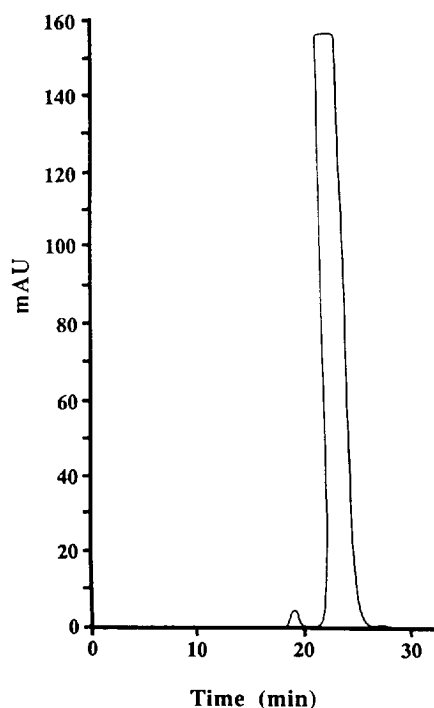
Fig. 4. HPLC of $\alpha$-anomer (retention time 24.0 min, area percent 99.2) after reversed-phase purification. Arafluoro-2-CdA (retention time 19.2 min, area percent 0.6) is present in this sample.

$[MH - C_5H_7O_3F]^+$ and $[MH - C_5H_8O_3F]^+$ cation. It was interesting to note that **3** showed a significantly greater yield of the $[MH - F]^+$ cation than **2**. The $^1H$ NMR spectra of **2** and **3** showed only one anomer which was unambiguously assigned. NOE difference experiments were used to assign the configurational preference about the C-1 carbon of the fluoro sugar (Fig. 1). Consideration of Dreiding model distances suggested that the H-4 proton of the 2-chloroadenine moiety could be used as a probe in an NOE difference experiment to assign **2** and **3**. When the H-4 proton of the 2-chloroadenine

moiety of **2** was irradiated, the H-1 and the H-3 protons of the fluoro sugar showed a strong NOE. These results are consistent with the 2-chloroadenine group being on the same face as the H-3 proton of the fluoro sugar. When the H-4 proton of the 2-chloroadenine moiety of **3** proton was irradiated, the H-1, H-2 and H-4 protons showed an NOE. These results are consistent with the 2-chloroadenine group being on the same face as the H-2 and H-4 protons of the fluoro sugar. The coupling constant between $C_1$-H and $C_2$-H for **3** was consistent with an $\alpha$-anomer, that is, all the couplings were large. The $C_2$-H proton for **3** appeared at approximately 5.62 ppm as a doublet of triplets with a geminal fluorine coupling of ca. 52 Hz.

## Acknowledgements

## References

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