Flash Chromatography

Chemistry 136

Flash chromatography, also known as medium pressure chromatography, was popularized several years ago by Clark Still of Columbia University, as an alternative to slow and often inefficient gravity-fed chromatography. Flash chromatography differs from the conventional technique in two ways: first, slightly smaller silica gel particles (250-400 mesh) are used, and second, due to restricted flow of solvent caused by the small gel particles, pressurized gas (*ca.* 10-15 psi) is used to drive the solvent through the column of stationary phase. The net result is a rapid ("over in a flash") and high resolution chromatography.

Selecting a Solvent System

The compound of interest should have a TLC R_f of ≈0.15 to 0.20 in the solvent system you choose. Binary (two component) solvent systems with one solvent having a higher polarity than the other are usually best since they allow for easy adjustment of the average polarity of the eluent. The ratio of solvents determines the polarity of the solvent system, and hence the rates of elution of the compounds to be separated. *Higher polarity of solvent increases rate of elution* for ALL compounds. Common binary solvent systems in order of increasing polarity are dichloromethane/hexane, ether/hexane, hexane/ethyl acetate, and dichloromethane/methanol. Hexane/ethyl acetate can be used on the bench, all other solvents should be used in the hood. If your R_f is a ≈0.2, you will need a volume of solvent ≈5X the volume of the dry silica gel in order to run your column.

Determining the Quantity of Silica Gel Required

The amount of silica gel depends on the R_f difference of the compounds to be separated, and on the amount of sample. For *n* grams of sample, you should use 30 to 100 *n* grams of silica gel. For easier separations, ratios closer to 30 : 1 are effective, for difficult separations, more silica gel is often required. However, by using more silica gel, the length of time required for the chromatography is extended. The density of powdered silica gel is about 0.75 g per mL.

Packing the Column

Obtain a glass column and make sure that it has either a glass frit or a plug of cotton wool directly above the stopcock to prevent the silica gel from escaping from the column through the stopcock. (IF it doesn't have either, you will have to put in a somewhat loosely stuffed plug of cotton wool; if you stuff it too much, solvent flow becomes painfully slow even with air pressure above the column). Next, put a ~1/2 in. layer of clean sand above the plug of glass wool. Use only as much as is necessary to obtain a flat surface, with the same diameter as that of the body of the column. Make sure the surface is flat. Then pour in the silica gel using a funnel. <u>DO THIS STEP</u>

IN THE HOOD! Silica gel is chemically similar to asbestos, and is a known carcinogen. Manipulations with dry silica should be done only in the hood.

Solvating the Silica Gel Column

Next, tap gently and evenly the sides of the column with a piece of rubber tubing to settle the silicagel. Pour a good amount of your elution solvent onto the silica gel. Use pressurized gas to force the solvent through the silica. As you force through a few hundred milliliters, you should see the top part of the silica become more homogeneous. This is because you are forcing out air that was entrapped in the silica gel. Continue to flush solvent through the silica gel until the entire silica plug becomes homogeneous in appearance. You may have to recycle the solvent coming through the column onto the top of the column several times before all the silica gel is solvated. Do not let the top of the column run dry, otherwise you will force air back into the top of the silica, and you will be back where you started.

Applying the Sample

Allow the solvent which remains above the silica to drain down until it is flush with the surface of the silica. If the top surface of the silica gel is not flat, gently tap the side of the column until it is. Dissolve your sample into the minimum volume of the elution solvent. Apply this to the top of the column, being careful not to disturb the top of the silica. Allow the sample to soak into the silica. Next, rinse the sides of the column with as **few** as possible milliliters of the elution solvent. Let this soak into the silica. After the rinsings have soaked into the silica gel, add a small amount of sand to protect the top surface of the silica when you add more solvent.

Eluting the Sample

Add a good part of your elution solvent to the column. Apply pressure to force the solvent through the column. The pressure should be the minimum necessary to keep a steady stream coming out of the column. Be aware that if you have chosen your solvent properly, it will take a little while before your compound of interest begins to elute. This means that the solvent, at first, contains none of your compound and can be discarded. If the R_f of your compound is 0.33 or less, you should be safe discarding an amount of solvent equal to the volume of the dry silica you used for the column. When you have collected this much solvent, begin collecting the eluted solvent into separate test tubes (fractions). When you have used all of your solvent, your sample should have finished eluting into the test tubes you collected. To maximize the efficiency of your chromatography, the fractions you collect should be no more than about one tenth of the column volume. For example, if you use 25 g of silica gel you should collect fractions of about 3 mL.

Locating the Sample

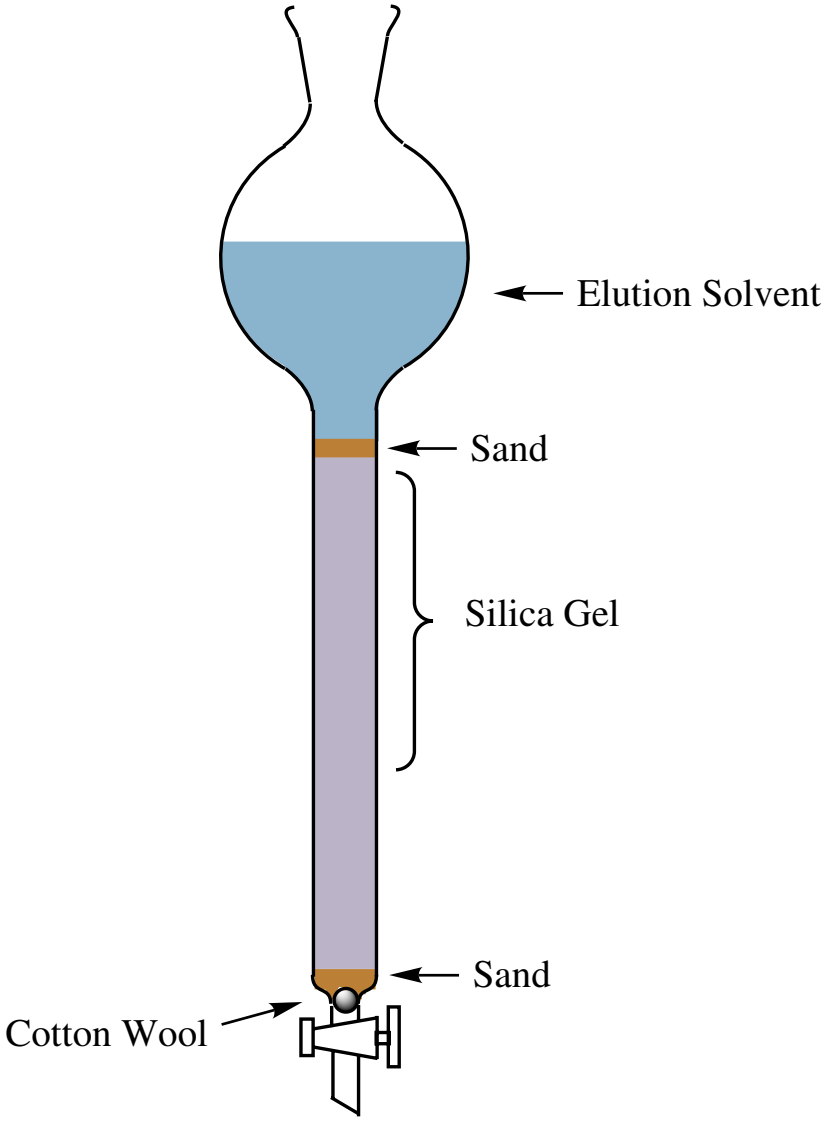
Use TLC to determine which fractions contain your compound. As the fractions fill, you should analyze each using TLC. It is **best to spot 10 fractions on one TLC plate and elute that 10 lane plate once**, rather than conducting individual analyses for each fraction. Combine the fractions that contain your sample together in a flask, then concentrate the sample on the rotavap (rotary evaporator).

Cleaning the Column

Flush all the remaining solvent out of the column using pressurized gas. When all liquid solvent has bee removed from the reservoir, remove the last remnants of solvent by applying a vacuum (from aspirator) to the bottom of the column.

Dispose of the used silica gel in a special collection container!!

Flash Chromatography Column



Original paper for Clark Still's

flash chromatography technique

Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution

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We wish to describe a simple absorption chromatography technique for the routine purification of organic compounds. Large scale preparative separations are traditionally carried out by tedious long column chromatography. Although the results are sometimes satisfactory, the technique is always time consuming and frequently gives poor recovery due to band tailing. These problems are especially acute when samples of greater than 1 or 2 g must be separated. In recent years several preparative systems have evolved which reduce separation times to 1–3 h and allow the resolution of components having $\Delta R_f \geq 0.05$ on analytical TLC. Of these, medium pressure chromatography[1] and short column chromatography[2] have been the most successful in our laboratory. We have recently developed a substantially faster technique for the routine purification of reaction products which we call flash chromatography. Although its resolution is only moderate ($\Delta R_f \geq 0.15$), the system is extremely inexpensive to set up and operate and allows separations of samples weighing 0.01–10.0 g[3] in 10–15 min.[4]

Flash chromatography is basically an air pressure driven hybrid of medium pressure and short column chromatography which has been optimized for particularly rapid separations. Optimization studies were carried out under a set of standard conditions[5] using samples of benzyl alcohol on a 20 mm × 5 in. column of silica gel 60 and monitoring the column output with a Tracor 970 ultraviolet detector. Resolution is measured in terms of the ratio of retention time (r) to peak width (w,$w/2$) (Figure 1), and the results are diagrammed in Figures 2–4 for variations in silica gel particle size, eluant flow rate, and sample size.

A number of interesting facts emerge from these data. First, we find that one of the most popular grades of silica gel 60, 70–230 mesh (63–200 μm), gives the poorest resolution of any gel studied under our standard conditions. Second, particle sizes less than 40 μm offer no improvement in resolution with our method of packing.[7] Column performance is quite sensitive to the rate of elution and is best with relatively high eluant flow rates. The solvent head above the adsorbent bed should drop 2.0 ± 0.1 in./min for optimum resolution with mixtures of ethyl acetate/petroleum ether (30–60 °C).[8] Finally, the peak width shows the expected increase with the sample size. Sample recovery was ≥95%.

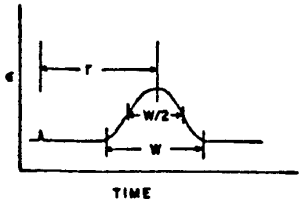


Figure 1. Typical chromatogram.

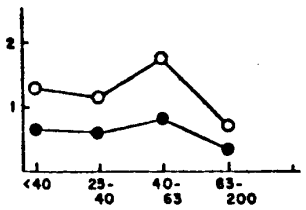


Figure 2. Silica gel particle size[6] (μm): (●) r/w; (○) $r/(w/2)$.

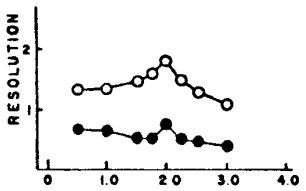


Figure 3. Eluant flow rate (in./min).

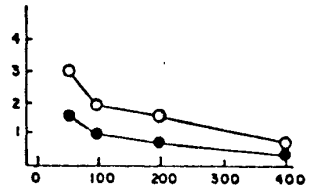


Figure 4. Sample size (mg).

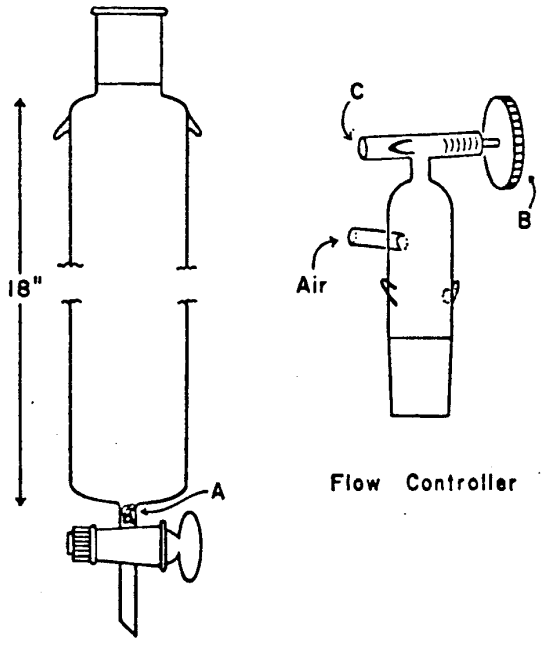


Figure 5.

The apparatus required for this technique consists of a set of chromatography columns and a flow controller valve (below). The column is a flattened bottom 18 in. glass tube fitted with a Teflon stopcock and topped with a 24/40 glass joint. Columns without fritted glass bed supports are generally preferred since they have significantly less dead volume than the standard fritted round-bottom variety. The flow controller

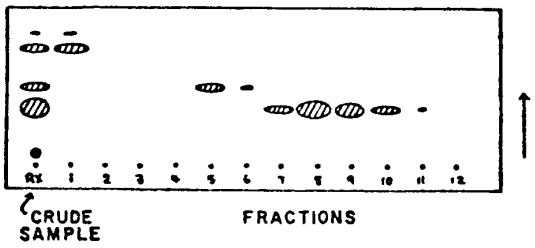


Figure 6.

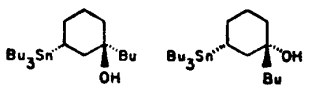
valve is a simple variable bleed device for precise regulation of the elution rate and is constructed from a glass/Teflon needle valve (Ace Glass Co. No. 8193-04 or equivalent) and a standard 24/40 joint.

A detailed procedure is presented in the experimental section and is summarized as follows: (1) A solvent is chosen which gives good separation and moves the desired component to R_f = 0.35 on analytical TLC (E. Merck No. 5765).[9] (2) A column of the appropriate diameter (see Table I) is selected and filled with 5–6 in. of dry 40–63 μm silica gel (E. Merck No. 9385).[10] (3) The column is filled with solvent and pressure is used to rapidly push all the air from the silica gel. (4) The sample is applied and the column is refilled with solvent and eluted at a flow rate of 2 in./min.

The time required to elute the desired components from the column is generally so fast (5–10 min) that we have abandoned automatic fraction collectors in favor of a simple rack holding forty 20 × 150 mm test tubes. Small fractions are typically collected early in the elution with larger ones being collected toward the end of the chromatography. Separated components are conveniently detected by spotting ~5 μL of each fraction along the long side of 7 cm × 2.5 cm TLC plate and then by developing the plate sideways. Heavier spotting may be required for small samples or highly retentive components. A typical separation is shown in Figure 6.

Over the past year we have run many hundreds of these columns. In every case we have been able to effect clean separation of compounds having $\Delta R_f \geq 0.15$ in less than 15 min and in many cases separations at $\Delta R_f \simeq 0.10$ were possible. The amount of sample used on a given column is proportional to its cross-sectional area and Table I can serve as a guide to column selection.

The sample size may increase substantially if less resolution is required; we have used a 50-mm column for the purification of up to 10 g of compound having impurities at $\Delta R_f \geq 0.4$. Resolution is maintained even with large diameter columns. For example the epimeric alcohols 1 and 2 have an R_f of 0.34



1 2

and 0.25, respectively, in 5% ethyl acetate/petroleum ether. A 1.0-g mixture of 1 and 2 (ΔR_f = 0.09) easily separated with only a 65-mg mixed fraction in 7 min on a 40-mm diameter column (500 mL of 5% EtOAc/petroleum ether).

If the components to be separated are closer on TLC than ΔR_f 0.15, increased resolution may be achieved by using a longer (e.g., 10 in.) column of gel alternatively a less polar solvent can be used. Such a solvent can be selected to move the desired components on TLC to R_f = 0.25 without increasing the elution times too drastically. In either case, the column should be only lightly loaded with sample and a rapid flow rate of 2 in./min should be maintained. Slower flows clearly give poorer resolution with ethyl acetate/petroleum ether mixtures.

Table I

column diameter, mm	vol of eluant,[a] mL	sample: typical loading (mg) $\Delta R_f \geq 0.2$	sample: typical loading (mg) $\Delta R_f \geq 0.1$	typical fraction size, mL
10	100	100	40	5
20	200	400	160	10
30	400	900	360	20
40	600	1600	600	30
50	1000	2500	1000	50

[a] Typical volume of eluant required for packing and elution.

In conclusion, flash chromatography provides a rapid and inexpensive general method for the preparative separation of mixtures requiring only moderate resolution. Even in cases where high resolution is required, preliminary purification by the flash technique allows simplified high-resolution separations without contamination of expensive HPLC columns. Finally, we would like to stress the facts that use of the 40–63 μm silica gel and a pressure- (and not vacuum-) driven flow rate of 2.0 in./min are crucial for successful separations by this method.

Experimental Section

Chromatography columns and the flow controller valve were assembled as described in the text. The silica gel used was 40–63 μm (400–230 mesh) silica gel 60 (E. Merck No. 9385).[10] Solvents were distilled prior to use. Thin layer chromatograms (TLC) were run on glass supported silica gel 60 plates (0.25-mm layer, F-254) (E. Merck No. 5765).

Flash Chromatography. General Procedure. First a low viscosity solvent system (e.g., ethyl acetate/30–60 °C petroleum ether)[8] is found which separates the mixture and moves the desired component on analytical TLC to an R_f of 0.35.[9] If several compounds are to be separated which run very close on TLC, adjust the solvent to put the midpoint between the components at R_f = 0.35. If the compounds are widely separated, adjust the R_f of the less mobile component to 0.35. Having chosen the solvent, a column of the appropriate diameter (see text, Table I) is selected and a small plug of glass wool is placed in the tube connecting the stopcock to the column body (A in the diagram above). Two telescoping lengths of glass tubing (6 and 8 mm o.d.) make placement of the glass wool plug easy. Next a smooth ⅛ in. layer of 50–100 mesh sand is added to cover the bottom of the column and dry 40–63 μm silica gel is poured into the column in a single portion to give a depth of 5.5–6 in. With the stopcock open, the column is gently tapped vertically on the bench top to pack the gel. Next a ⅛ in. layer of sand is carefully placed on the flat top of the dry gel bed and the column is clamped for pressure packing and elution. The solvent chosen above is then poured carefully over the sand to fill the column completely. The needle valve (B) of the flow controller is opened all the way and the flow controller is fitted tightly to the top of the column and secured with strong rubber bands. The main air line valve leading to the flow controller is opened slightly and a finger is placed fairly tightly over the bleed port (C). This will cause the pressure above the adsorbent bed to climb rapidly and compress the silica gel as solvent is rapidly forced through the column. It is important to maintain the pressure until *all* the air is expelled and the lower part of the column is cool; otherwise, the column will fragment and should be repacked unless the separation desired is a trivial one. Particular care is necessary with large diameter columns. The pressure is then released and excess eluant is forced out of the column above the adsorbent bed by partially blocking the bleed port (C). The top of the silica gel should not be allowed to run dry. Next the sample is applied by pipette as a 20–25% solution in the eluant to the top of the adsorbent bed and the flow controller is briefly placed on top of the column to push all of the sample into the silica gel.[11] The solvent used to pack the column is ordinarily reused to elute the column. The walls of the column are washed down with a few milliliters of fresh eluant, the washings are pushed into the gel as before, and the column is carefully filled with eluant so as not to disturb the adsorbent bed. The flow controller is finally secured to the column and adjusted to cause the surface of the solvent in the column to fall 2.0 in./min. This seems to be an optimum value of the flow rate for most low viscosity solvents for any column diameter with the 40–63 μm silica gel. Fractions are

collected until all the solvent has been used (see Table I to estimate the amount of solvent and fraction size). It is best not to let the column run dry since further elution is occasionally necessary. Purified components are identified as described in the text by TLC. If the foregoing instructions are *followed exactly, there is little opportunity* for the separation to fail.

Although we generally pack fresh columns for each separation, the expense of large-scale separations makes it advantageous to reuse large diameter columns. Column recycling is effected by first flushing (rate = 2 in./min) the column with approximately 5 in. of the more polar component in the eluant (generally ethyl acetate or acetone) and then with 5 in. of the desired eluant. If the eluant is relatively nonpolar (e.g., ≤10% EtOAc/petroleum ether), it may be more advisable to use a flushing solvent (e.g., 20–50% EtOAc/petroleum ether) which is somewhat less polar than the pure high polarity component.

Registry No.—1, 66417-28-5; 2, 66417-27-4.

References and Notes

(1) Such units have been described and used extensively by J. M. McCall, R. E. TenBrinkt, and C. H. Lin at the Upjohn Company and A. I. Meyers at Colorado State University.
(2) B. J. Hunt and W. Rigby, *Chem. Ind. (London)*, 1868 (1967).
(3) *This is not a limitation but is merely the scale range which we have used.*
(4) This is the total time required for column packing, sample application, and complete elution.
(5) Standard conditions: 5 in. high bed of 40–63 μm silica gel 60 in a 20 mm diameter column packed as described in text, 2.0 in. of solvent flow/min, 200 mg of benzyl alcohol, 25% ethyl acetate/petroleum ether eluant.
(6) These gels are manufactured by E. Merck and are the following grades: <40 μm (silica gel H, No. 7736), 25–40 μm (LiChroPrep Si60, No. 9390), 40–63 μm (silica gel 60, No. 9385), 63–200 μm (silica gel 60, No. 10180).
(7) Slurry packing, incremental dry packing, or single portion dry packing gave identical results with the 40–63 μm gel. Since the last technique was the simplest, it was employed in all our studies.
(8) This is a particularly good general solvent system. For extremely polar compounds, acetone/petroleum ether or acetone/methylene chloride mixtures are often useful. Significantly higher viscosity solvents will require slower optimum resolution flow rates.
(9) If this R_f is given by a solvent having <2% of the polar component, a slightly less polar eluant is desirable. Thus if 1% ethyl acetate/petroleum ether gives a compound an R_f of 0.35 on TLC, the column is run with 0.5% ethyl acetate.
(10) 40–63 μm gel is also used for medium pressure chromatography[1] and is available from MCB in 1 kg ($45/kg) or 25 kg ($16/kg) lots.
(11) If the sample is only partially soluble in the eluant, just enough of the more polar component is added to give complete dissolution. Large quantities of very polar impurities are best removed prior to chromatography so that excessive quantities of solvent or large increases in solvent polarity will be unnecessary for sample application.

Homo-*C*-nucleosides. The Synthesis of Certain 6-Substituted 4-Pyrimidinones[1]

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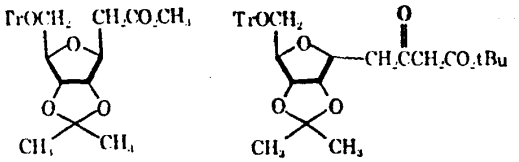
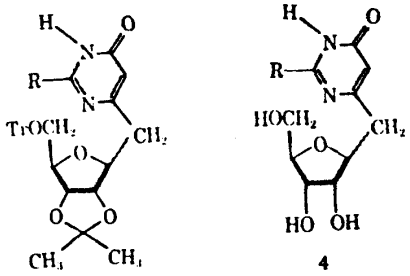
The chemistry of *C*-nucleosides has received considerable attention recently due to the biological activities of naturally occurring compounds such as showdomycin, formycin, and oxazinomycin.[2] Though synthetic methodology has evolved for the preparation of a number of *C*-nucleoside analogues,[2] only one investigation has dealt with the synthesis of homo-*C*-nucleosides,[3] compounds with a methylene unit between a carbon of the nitrogen base and the standard D-ribose moiety. This note describes the facile synthesis of a series of 6-substituted 4-pyrimidinone homo-*C*-nucleosides from the ester 1, which is available in three steps from D-ribose.[4,5]

Treatment of 1 with lithio-*tert*-butyl acetate[6] in toluene at 0 °C for several hours affords an anomeric mixture (ca. 3:1, β/α) of the β-keto ester 2 in 75% yield. The assignment of β to the major anomer was made on the basis of ^{13}C NMR data. In particular, the isopropylidene methyls of the major anomer occur at δ 25.66 and 27.54, within the range strongly indicative of a β configuration (25.5 ± 0.2 and 27.5 ± 0.2).[7,8]



1 2

Tr = trityl



3 4

a, R = NH_2
b, R = CH_3
c, R = SH
d, R = phenyl
e, R = H

It has been shown that the α-anomer of 1 is more stable than the β,[4] and recently a rationalization for this seemingly unusual behavior has been presented.[9] On this basis it seems likely that the α anomer of 2 is also more stable than the β. The conditions involved in the preparation of 2 (low-temperature, aprotic solvent) probably do not allow equilibration, though there is some leakage to the α-anomer. Further support for these postulates is provided by the finding that β-2 is isomerized readily under basic conditions to an α/β mixture which is predominantly α.

Condensation of 2 with guanidine, acetamidine, thiourea, and benzamidine under basic conditions afforded the protected nucleosides 3a–d as anomeric mixtures (ca. 3:1, α/β) which were chromatographically inseparable. That the major anomers after condensation are all α is also indicated by the chemical shifts of the isopropylidene methyls. For example, the shifts of the methyls in 3a are at δ 25.09 and 26.33, clearly in the α range (24.9 ± 0.3 and 26.3 ± 0.2).[7,8] In view of the ready isomerization of β-2 to a mixture of anomers containing predominantly α-2, it seems likely that equilibration is occurring prior to cyclization, and that the anomeric composition of 2 after equilibration dictates the ratio of α- and β-homo-*C*-nucleosides. Desulfurization of 3c with Raney Nickel in refluxing 95% ethanol provided the hydrogen-substituted compound 3e. Interestingly, while both urea and formamidine reacted with 2, neither led to the formation of cyclized material under a variety of conditions. The free nucleosides 4a–e were obtained by treatment of 3a–e with either methanolic hydrogen chloride or aqueous trifluoroacetic acid for several hours. These acidic conditions, even over longer periods of time (2 days), caused no change in the α/β ratio of the nucleosides. Chromatographic separation of the free nucleoside anomers was once again not possible. 4e was also available by desulfurization of 4c.

The ^{13}C NMR spectra of the free nucleosides contained characteristic signals for the five compounds, and all values are reported in the Experimental Section. Salient ^1H NMR values are the methyl singlet of 4b at δ 2.28 and the pyrimidine C_2H singlet of 4e at δ 8.92, as well as the pyrimidine C_5 signal of all five nucleosides in the neighborhood of δ 6.0.