Purpose: To investigate alternative chrom. procedures to the ones currently being used for adduct chromatography.

Procedure

Preparation of Adduct Standards

Take the ethanol-2 phase from J-1 (2/3 STOH) and remove the ethanol. (This is an enzymatic hydrolysate)

Vol. taken: 2.5 ml

Vol. remaining after rotary evaporation: 5.1 ml

Sample 6-5-1

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6-5-76 1. **Gradient Conditions:**

RAnget Column, 10% MeOH → [60% of 80%] in 30 min.

Results: adduct peak elutes at 25 min - it is pretty symmetrical with a definite small peak coming off the tail.

6-6-76 2. Conditions:

10% MeOH → [45% of 80%] in 30 min.

Peak 1 elutes at 27 minutes; better resol. from the trailing peak

3. Conditions:

10% MeOH → [30% of 80%] in 30 min

Peak 1 elutes at 35.5 minutes
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Observation: The timer controlling the length of the gradient is not working correctly. The 30 min gradient takes much longer than that (more than 40 min).

Adduct Chromatography on Waters C-18 Column

Column: 30 cm Waters µ-C18
Conditions: T = 54°C, P = 800-1000 psi, N=1.0
30 min gradient, 10% MeOH → 80% MeOH (linear)
Results:
Excellent resolution - injected 40 µl 6-5-1
The adduct peak is needle-sharp and appears to be pure. Also, it comes out at a time (29 min) which is at the very beginning of the gradient rise, so I should be able to get relatively pure material.

Note: a second injection did not look as good - 100 µl was injected and the column may have been overloaded.

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Nucleoside/Base Retention Characteristics on Waters C18

The (4) DNA bases and nucleosides were injected together. Individual retention times were not obtained.