Adduct 12-15-99

$$\frac{3019\ cpm}{.03\ ml} = 1.006 \times 10^5\ cpm/ml \times 12.9\ ml = 1.298 \times 10^6\ cpm$$

Adduct 12-15-100

$$\frac{3515.8\ cpm}{.03\ ml} = 1.172 \times 10^5\ \frac{cpm}{ml} \times 13.6\ ml = 1.594 \times 10^6\ cpm$$

12-15-76 Preparative Chromatography on Sample 12-15-1

Total volume which must be processed: 135 ml

1. SAMPLE 12-15-99 ADDUCT

- 15 ml of 12-15-1 pumped onto PC at S=30
- rinse graduate with 3 ml 10%
- pump on 10% for 10 min
- connect to micromeritics and pump at 3 ml/min for 10 min
- start 40 min gradient
- collect adduct at ~ 28-30 min (fract. coll at 4 min intervals along the way).

volume of 12-15-99 : 12.9 ml

counts: 12,076 / 4 = 3019/30 µl

total amount of adduct : 1.298×10^6 cpm

2. Sample 12-15-100 ADDUCT

- same conditions, except
  - 20 ml of 12-15-1
  - rinse with 10% till 254 ↓ (lot of DNA)
  - run 45 min gradient - better resolution - may even want to go longer with larger quant. DNA

volume of 12-15-100 : 13.6 ml

Count 30 µl : 14,063 cp / 4 min = 3515.8 cpm

total amount of adduct : 1.594×10^6 cpm

12-16-1 Adduct

11699 cpm / 0.1 ml = 1.17×10^5 cpm/ml

× 15.6 ml ⟶ 1.825×10^6 cpm total

12-16-2 Adduct

15074 c / 4 min = 3768.5 cpm

30 µl

⇒ 1.256×10^5 cpm/ml $\xrightarrow{\times 16}$ 2.01×10^6 cpm total

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12-16-3 Adduct

1.486×10^4 C / 4 min / .03 ml ⟶ 1.239×10^5 cpm/ml

$\xrightarrow{\times 16.5}$ 2.044×10^6 cpm total

Tally Sheet on Adduct Isolated

Run	cpm x 10^6	mg
1.	1.298	
2	1.594	
3	1.825	
4	2.010	
5	2.044	
6	2.006	
7	1.212	

TOTAL = 12.04×10^6 cpm $\xrightarrow{\cdot 33}$ 36.4

2.2×10^6 dpm/µCi ⟶ 16.55 µCi

$\frac{16.55\ \mu Ci}{1.52\ \frac{\mu Ci}{\mu mole}} = 1.089\ \mu mole \times 479\ \frac{\mu g}{\mu mole}$

⟶ 5.216 mg ⇒ 2308 $\frac{cpm}{\mu g}$

AMOUNT OF ADDUCT ISOLATED: 5.2 mg

12-16-76 Sample: 12-16-1 ADDUCT 1.52 μCi/μM

3. 20ml of 12-15-1

45 min gradient by Paul Donalue. No problems. 46,796 c/4 min for 100μl. Total fraction volume = 15.6 ml.

total quantity of adduct: 1.825×10^6 dpm

Sample 12-16-2 ADDUCT

4. 20ml of 12-15-1 (PRD)

Volume: ~16 ml
radioactivity: 15,487 /4 min / 30μl
14,661

5. Sample 12-16-3 ADDUCT (PRD)
Volume: 16.5 ml
15519 /4 min / 30μl
14498
14573

Runs 1-5 → 8.77×10^6 dpm $\xrightarrow{\div .33}$ 2.66×10^7 dpm $\xrightarrow{2.22 \times 10^6 \text{ dpm}/\mu\text{Ci}}$ 1.20 μCi

SA = 1.52 μCi/μM ∴ → 0.788 μmoles adduct

There are 479 μg/μmole ∴ → 3.77 mg adduct