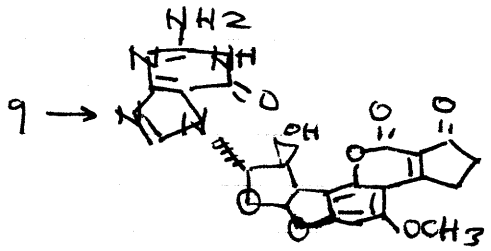
22-76 Development of Conditions for Adduct Methylation

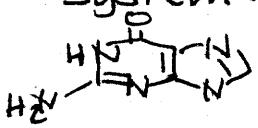
Purpose: We have good MS and NMR Data on the adduct, and it is all consistant with substitution either on the N^3, N^9 or most likely the N^7 of guanine:

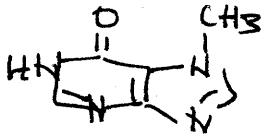


In this case the N^9 is open and available for alkylation — If 9-Methylguanine is obtained, then it is pretty good evidence that the original adduct was bound to the N^7 position (excluding the possibility of 9,9 disobstitution)

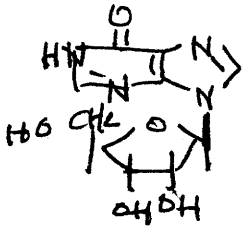
Procedure:

First test these model compounds in an alkylating System:


guanine



7-MeGua



G

- put ~ 50 mg each into PS vials
- add 250µl dimethylacetamide
- heat to 150°C
- G dissolved completely, the others didn't add 250 µl DMA to 7MG + Gua
- ~~filter~~ Centrifuge down solids and take SN
- add 40µl DIMETHYLSULFATE
- heat 10 min at 130°C
- cool in ice and add another 40 µl DMA
- Continue heating to a total of 2 hrs.
- cool samples in ice
- add 8-9 drops NH_4OH to make it alkaline (~pH8)
- add 200µl MeOH.

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Obs. The Gua sample turned turbid after base addition – The Gua and the 7-MeGua didn't, even after MeOH addition.

- samples stored in ~~refrigerator~~ freezer.

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- Inject the Gua methylation product into DC4A system
- no peak at t_R Gua – lots of garbage at beginning
- there were pump/injector problems, but I don't think the compound came out at late t_R

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Hydrolysis of Synthesized M^7G

- The sample of guanosine methylated with DMS (200µ
was evaporated to ~ 15µℓ with N_2 at 57°
- hydrolyze with 100µℓ $HClO_4$
Obs: 2-phase system formed
- heat 100° 1hr → a ppt formed!
- next. 470µℓ KOH (3N) + 1 drop HCOOH
- inject 20 µℓ into HPLC (ion exchange) → garbage at front, no peak where 7-MeGua eluted.

* It is possible that the imidazole ring opened when I made the samples alkaline.