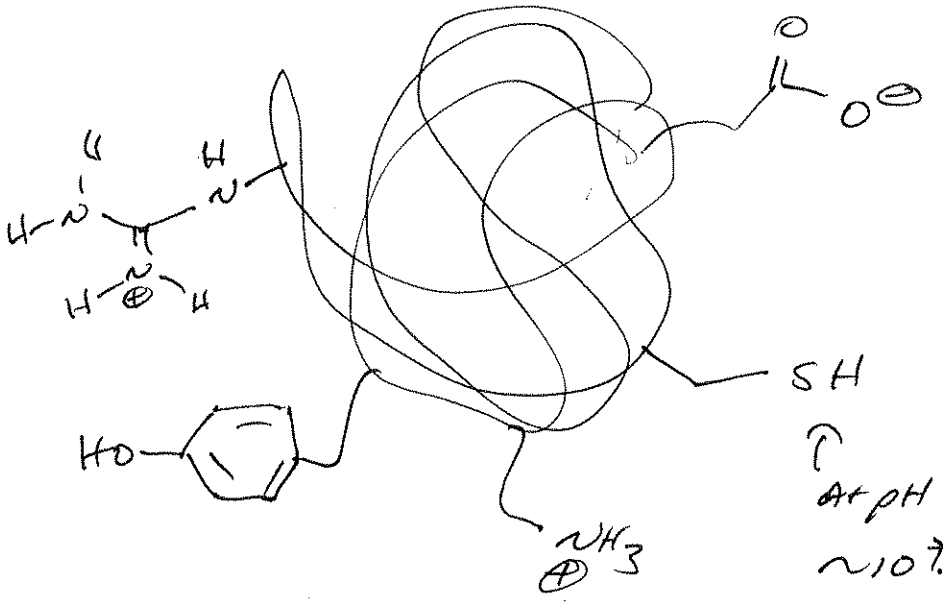
Answer key – Problem Set 1

1. pH 7



At pH 7, thiol is ~10% as $RS^{\ominus}$, ~90% RSH

If pH < pKa, then major form is acidic (conditions are acidic for that acid)
↑
conditions

If pH > pKa, then major form is conjugate base (conditions are basic for that acid)

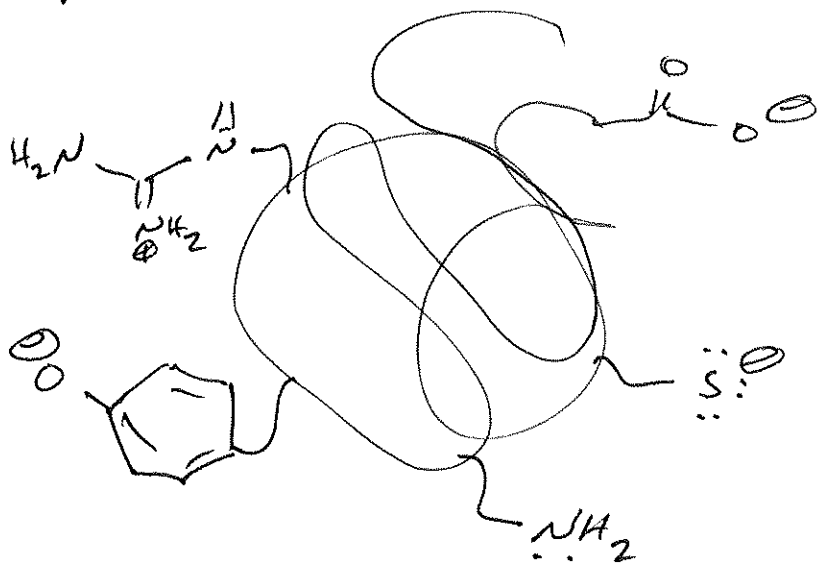
pKa = pKa at which functional group is half in acidic form, half in conjugate base.

pH / pKa are log base 10

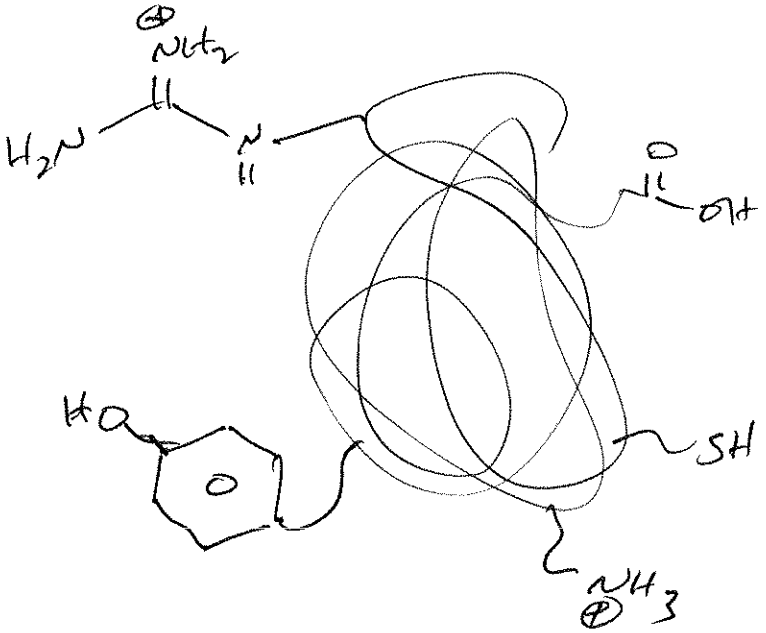
∴ Each pH unit away from pKa is a factor of 10 difference in relative amounts

e.g. RSH
pH 7 10:1 acidic : basic
pH 6 100:1 acidic : basic
pH 10 1:100 acidic : basic

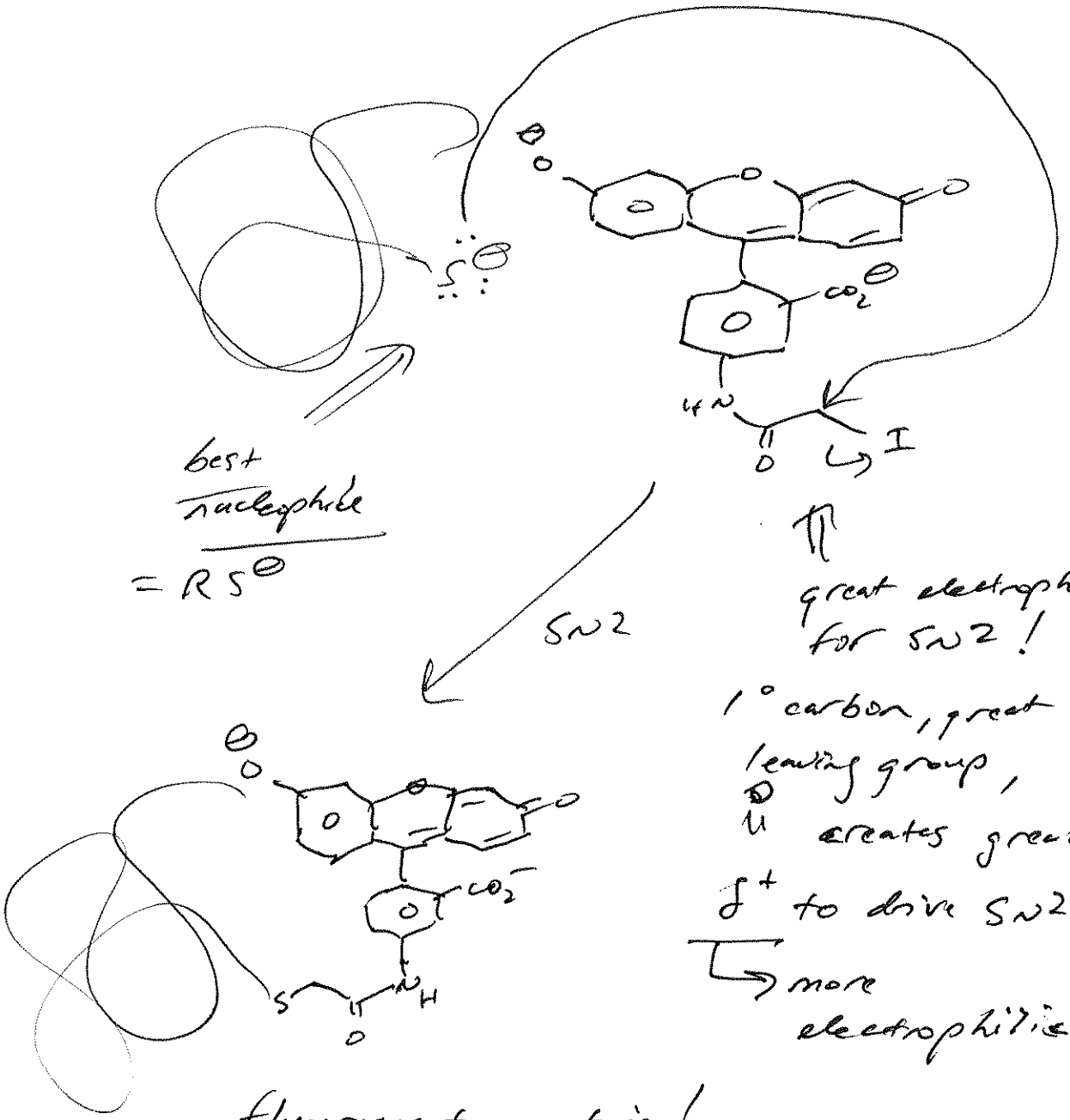
2. pH 11



3. pH 3



4.



fluorescent protein!

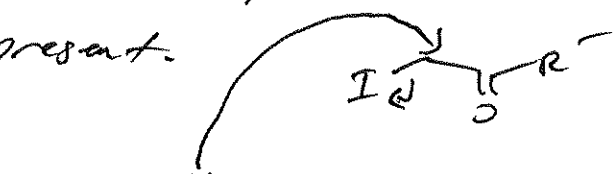
Note: 1) This reaction works best at pH 7-8.

$$RSH \rightleftharpoons RS^{\ominus} \xrightarrow{S_N2} RS\text{-}\underset{\underset{O}{\|}}{C}\text{-}R'$$

much better nucleophile

Need thiolate form for reaction to occur rapidly. Le Chatelier's principle results in rapid labeling of 100% of protein on 1 site.

5. At pH 11, 2 additional nucleophiles are present.



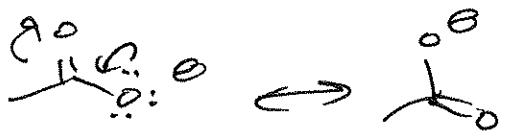
~ $\ddot{N}H_2$ excellent nucleophile

good nucleophile

So get SN2 products from these (especially Lysine, one of the most common amino ? $R\overset{\oplus}{N}H_3$ acids.) Will observe mixture of products due to labeling on $RS^{\ominus}$, on RNH_2, and on both. (Some fraction will have labeling on Tyr.)

In practice, these protein labeling reactions should be done at pH ≤ 8. At pH > 8, enough Lysine is $R\ddot{N}H_2$ that you get observable side reactions on lysine in addition to the desired reaction on Cysteine (RSH/ $RS^{\ominus}$).

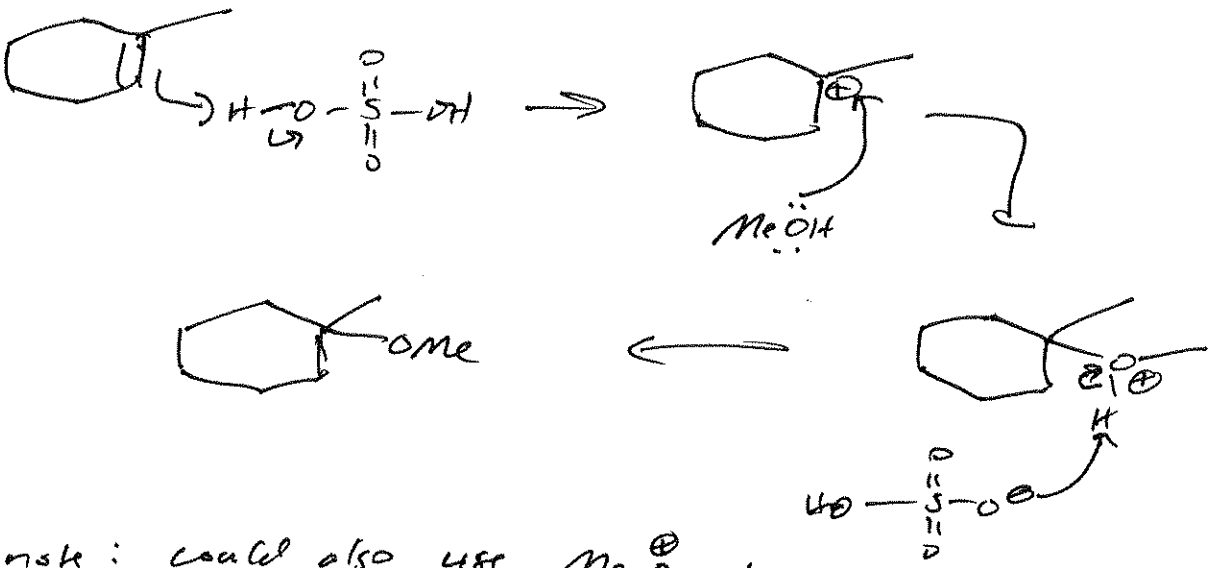
6. pH 5: Molecule has only 1/1000 thiols in thiolate form. Since S_N2 rate law is rate = $k[R-I][\text{nucleophile}]$, the rate is dependent on [nucleophile], here $[RS^{\ominus}]$. If total protein [ ] is 1 mM, then at pH 5 only 1/1000 (= 1 μM) is $RS^{\ominus}$, and the reaction rate is ~1000× slower than when protein is mostly in $RS^{\ominus}$ form. These data also remind us that $R-\overset{\overset{O}{\|}}{C}-O^{\ominus}$ is a much weaker nucleophile than $RS^{\ominus}$. In practice, you do not see S_N2 reaction in H_2O with this nucleophile except with exceedingly good ~~nucleophiles~~ electrophiles.



Delocalization of e⁻ also reduces nucleophilicity

(Note: $R-\overset{\overset{O}{\|}}{C}-S^{\ominus}$ and RSH are similarly good nucleophiles. At higher temp and/or with time, both will react. However, rxn. w/ $RS^{\ominus}$ is complete in 5–15 minutes at pH 8.)

7.



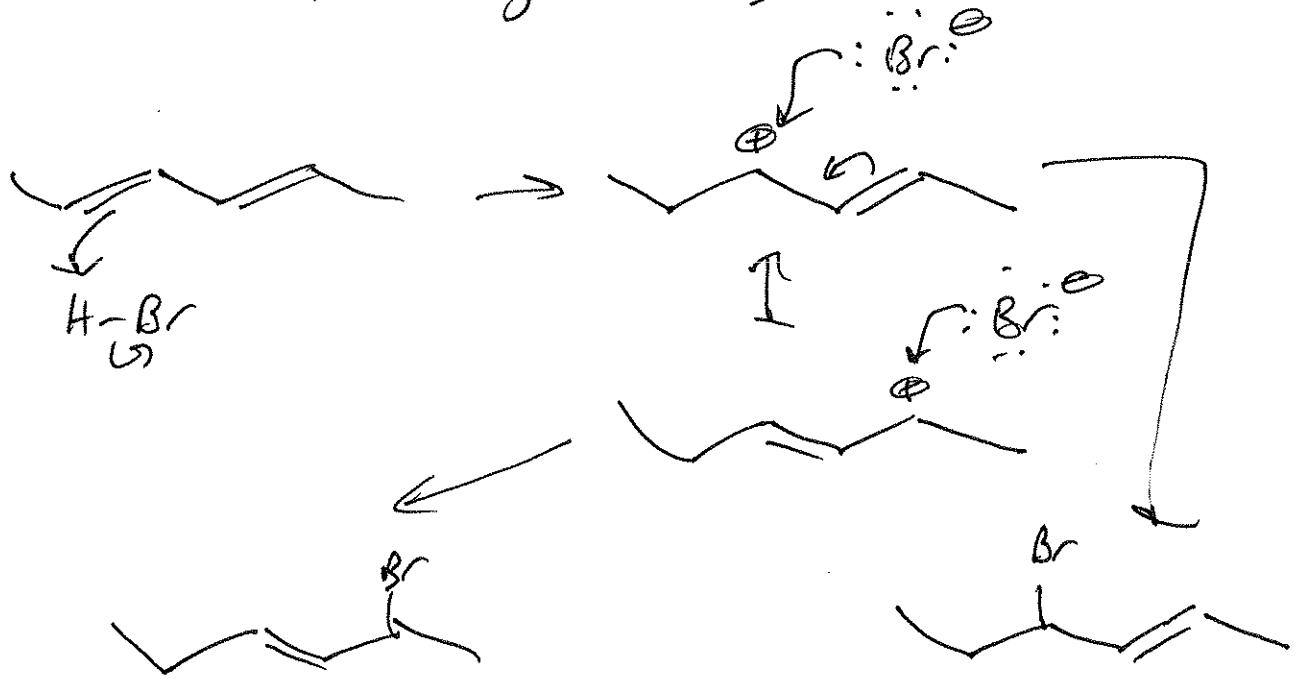
note: could also use $MeO^{\oplus}(H)-H$ as acid (pKa −3) (like hydronium)

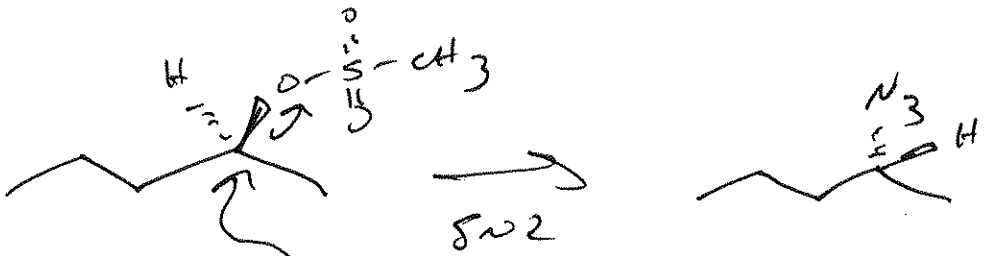
(formed $H_2SO_4 + MeOH \rightleftharpoons HOSO_4^- + MeOH_2^{\oplus}$)

Could also use $MeOH$ as base.

Cannot use MeOH as acid (too weak, pKa 15)

Cannot use $MeO^{\ominus}$ as base/nucleophile (cannot exist in strong acid)

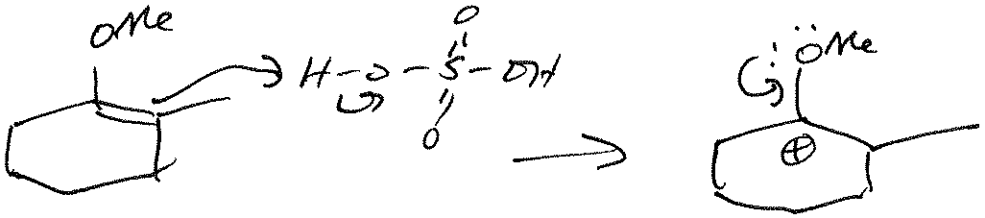


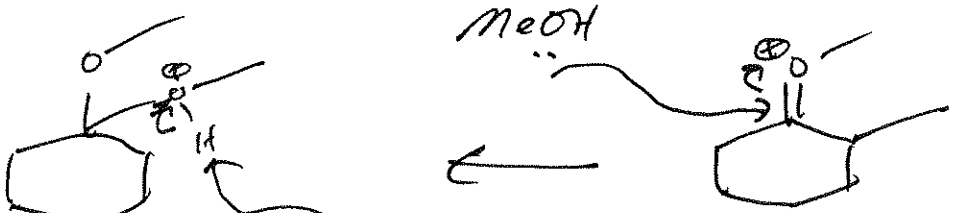


excellent nucleophile ($^{\ominus}N_3$)

$^{\ominus}O-\overset{O}{\overset{\|}{S}}-CH_3$ excellent leaving group

(conjugate base of strong acid)





highly stabilized carbocation

(resonance > 3°)

↑ extra bond here!