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— First test on SPAAC (DBCO-Apt, LNO@Talys)

1) Modification of NH_2 -Aptamer with DBCO-NHS:

0.1 mM apt- NH_2 (aliquot in freezer)
100 μM

Modify per column: 0.5 nmol (5 μL aliquot)

5 eq DBCO-NHS: 2.5 nmol $\rightarrow$ (MW: 532.5 g/mol)

Modification all together: in 1 NH_2 -Bov binding dependent.

1) 40 μL of apt. aliquot (NH_2 -Apt)

+ 10 μL of DBCO-NHS: 0.266 mg/mL in PBS pH 8.3
(5 eq DBCO $\rightarrow$ 5 nmol) $\rightarrow$ 2.66 μg

Want to have 2.66 μg in 10 μL $\rightarrow$ 0.266 mg/mL

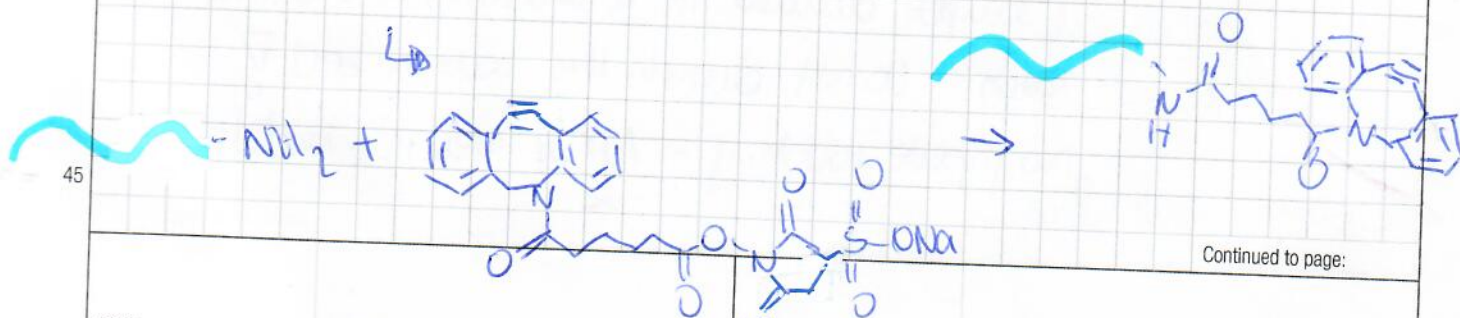
Stirring 2h, rt

2) After 2h: Add 20 μL PBS pH 9.3

+ other 5 eq DBCO-NHS (30 μL of sol)

Stirring 2h, rt : tot vol: 50 μL

After 2h, excess DBCO-NHS was removed with size-exclusion column (MicroSpin G 50 col) $\rightarrow$ pre-equilibrated with water + 0.1% NaOH



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