Supporting Information

Syntheses of Regioisomerically Pure 5- or 6-Halogenated Fluoresceins

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Contents

- general procedures
- a procedure to obtain 5-iodo-2-sulfobenzoic anhydride
- ^1H and ^{13}C NMR spectra for **1a**, **2a**, **3**, **4**, **9a**, **9b**, **10a**, and **11**

General Methods. High field NMR spectra were recorded on Varian Unity Plus (^1H at 300 MHz, ^{13}C at 75 MHz) or Inova (^1H at 500 MHz, ^{13}C at 125 MHz) NMR spectrometers. Mass spectra were obtained from the Mass Spectrometry Applications Laboratory at Texas A&M University. 5-Amino and 6-aminofluoresceins were purchased from Fluka Co. or prepared following a literature procedure.² 5-Iodosulfobenzoic anhydride¹² was prepared according to literature procedure with a slight modification.¹³ 5-Aminofluorescein diacetate¹¹ and 6-aminofluorescein diacetate¹⁰ were prepared according to literature procedures. Other chemicals were used as purchased from chemical suppliers.

5-Iodo-2-sulfobenzoic anhydride. Sodium 5-iodo-2-sulfonbenzoate (10 g, 28.6 mmol) was placed in a 50 mL round-bottomed flask. Thionyl chloride (21 mL, 286 mmol) was added all in one portion. The mixture was refluxed under N_2 for 7 h. After cooling to room temperature, thionyl chloride was distilled off with a tube connected to one of the exit of the receiver and passed into NaOH solution to carry off HCl and SO_2 generated during the distillation. Then 100 mL of benzene was added and boiled for 1 h; some white precipitate formed. The hot mixture was filtered. The solid was extracted with hot benzene (50 mL X 2). The combined filtrates were then condensed in vacuo until 20 mL of benzene remained. Then the solution was cooled in an ice bath. 5-Iodosulfobenzoic anhydride precipitated out as an off-white solid that was recrystallized from benzene (4.15 g, 47 % yield). ^1H NMR (300 MHz, CDCl_3): δ 8.36 (dd, $J = 1.2, 0.5$ Hz, 1H), 8.30 (dd, $J = 8.1, 1.2$ Hz, 1H), 7.84 (dd, $J = 8.1, 0.5$ Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3): δ 154.9, 144.8, 141.3, 131.4, 128.6, 124.7, 105.3; MS (ESI+TOF) m/z 311 ($\text{M}+\text{H}$)⁺.

STANDARD PROTON PARAMETERS
Pulse Sequence: zgpg30
Solvent: DMSO
Acquired Temperature:
INSTRUMENT: spect
Pulse: zgpg30
Acq. time 2.812 sec
INSTRUMENT: spect
ORIGIN: 11.500, 1479125 MHz
DATA PROCESSING
FT size 65536
Total time 8 min, 58 sec

¹H NMR

STANDARD 14 RESERVE
 Pulse Sequence: zgpg30
 Solvent: dmso
 Concent: 100000000
 Name: 2a
 Relax: delay 1.000 sec
 Pulse: 50.0 degrees
 Acquire: 4.000 sec
 Width: 6586.5 Hz
 F2: 700.151200
 Offset: 0.000000
 Data: 6586.515120
 F1: 100.626150
 Total Time: 1 min, 3 sec

¹H NMR

STANDARD 1H NMR
 Pulse Sequence: zgpg30
 Solvent: CDCl3
 Acquisition Temperature: 300.2 K
 Orbitals: 388 "unit3000"
 F2: 400.136 MHz
 Aq: 1.00000000
 Width: 4000.000 Hz
 3 repetitions
 298.15 K
 298.15 K
 298.15 K
 Total time: 9 min, 11 sec

¹H NMR

STROBILIN: CARBON PARAMETERS
Pulse Sequence: zgpg30

^{13}C NMR of 9b

^{13}C NMR of
6-Bromofluorescein
diacetate

STANDARD CARBON PARAMETERS

 ^{13}C NMR of 11

STANDARD IN E66294E
 Pulse Sequence: zgpg30
 Solvent: DMSO
 Acquisition Temperature: 300.2 K
 F1 (MHz): 400.146399
 INSTR: zgpg30
 Relax. delay: 1.00 sec
 Pulse: 63.0 deg
 Acq.: 1.00 sec
 F2 (MHz): 100.628155
 F2 (ppm): 100.628155
 OBSERVE: 1H
 DATA PROCESSING
 FT size: 32768
 Total time: 1 min, 9 sec

¹H NMR