

# Human Serum Albumin Labelling with a New BODIPY Dye Having a Large Stokes Shift

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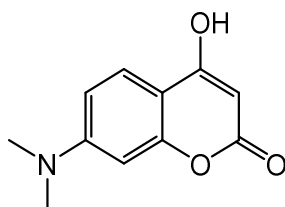
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\* Correspondence: [abramova@niboch.nsc.ru](mailto:abramova@niboch.nsc.ru); Tel.: +7-383-363-51-83 (T.V.A.)

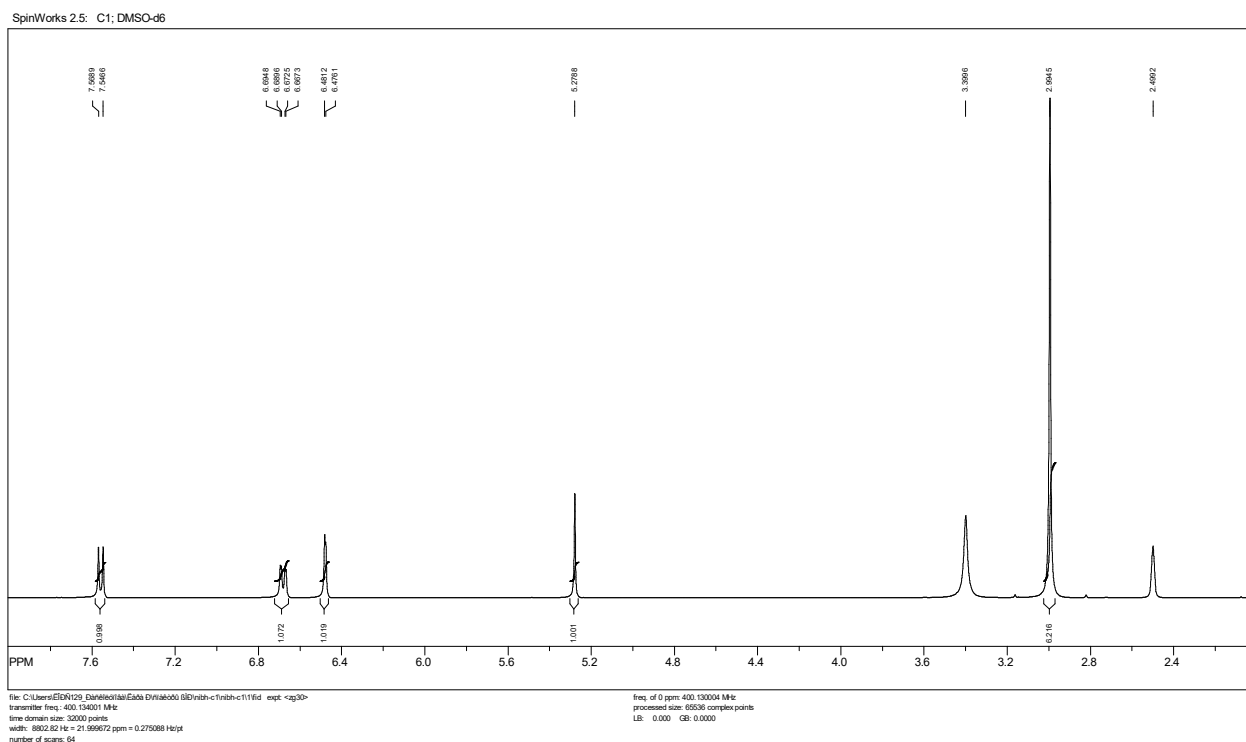
## Synthetic protocols and spectral data for new compounds

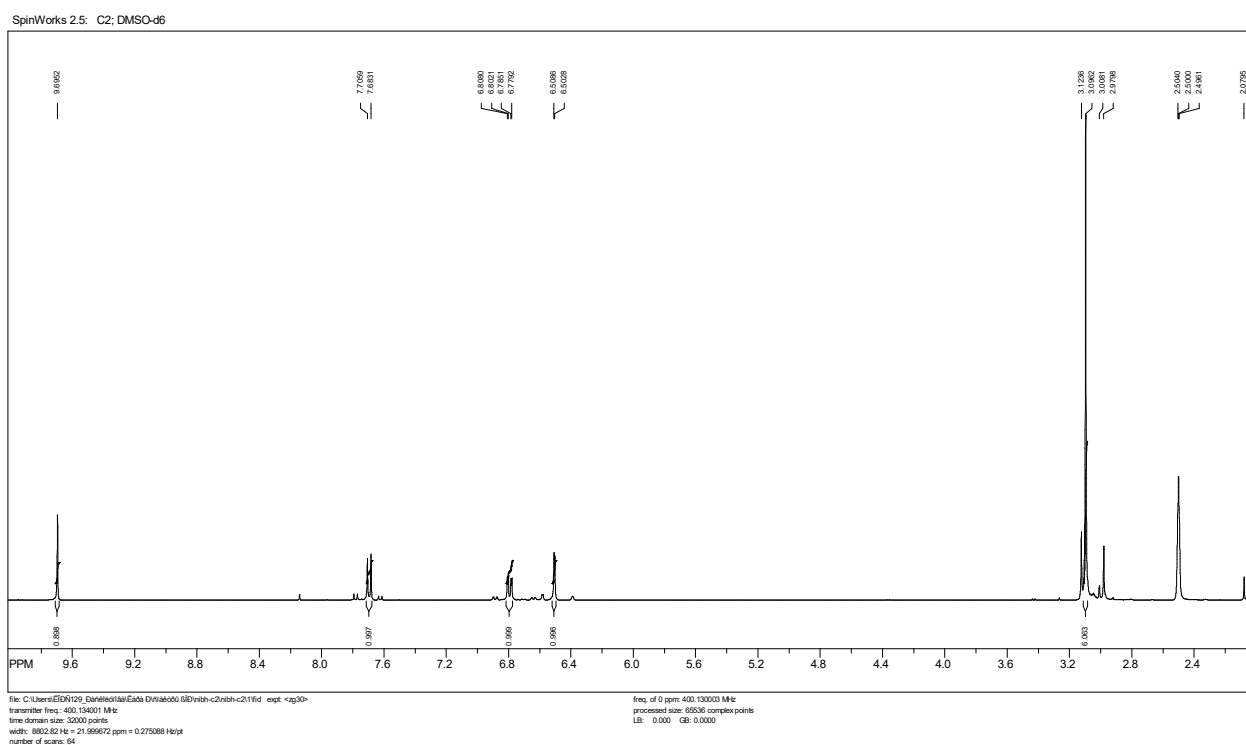
### 7-(Dimethylamino)-4-hydroxy-2H-chromen-2-one (3)



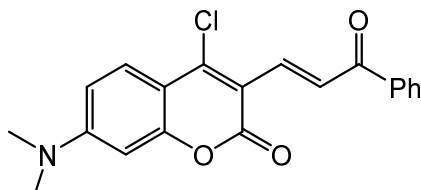
Solution of diphenyl malonate (2.36 g, 10 mmol) and 3-(*N,N*-dimethylamino)phenol (1.52 g, 16 mmol) in toluene (30 mL) was refluxed for 10 h. The reaction mixture was cooled and crystals formed were filtered off. The crude product was recrystallized from ethanol. Yield: 1.84 g, 9.0 mmol, 89%. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 7.56 (1H, d, *J* 8.9, H5), 6.68 (1H, dd, *J* 9.0, 2.2, H6), 6.48 (1H, d, *J* 2.1, H8), 5.28 (1H, s, H3), 2.99 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>).

### <sup>1</sup>H NMR



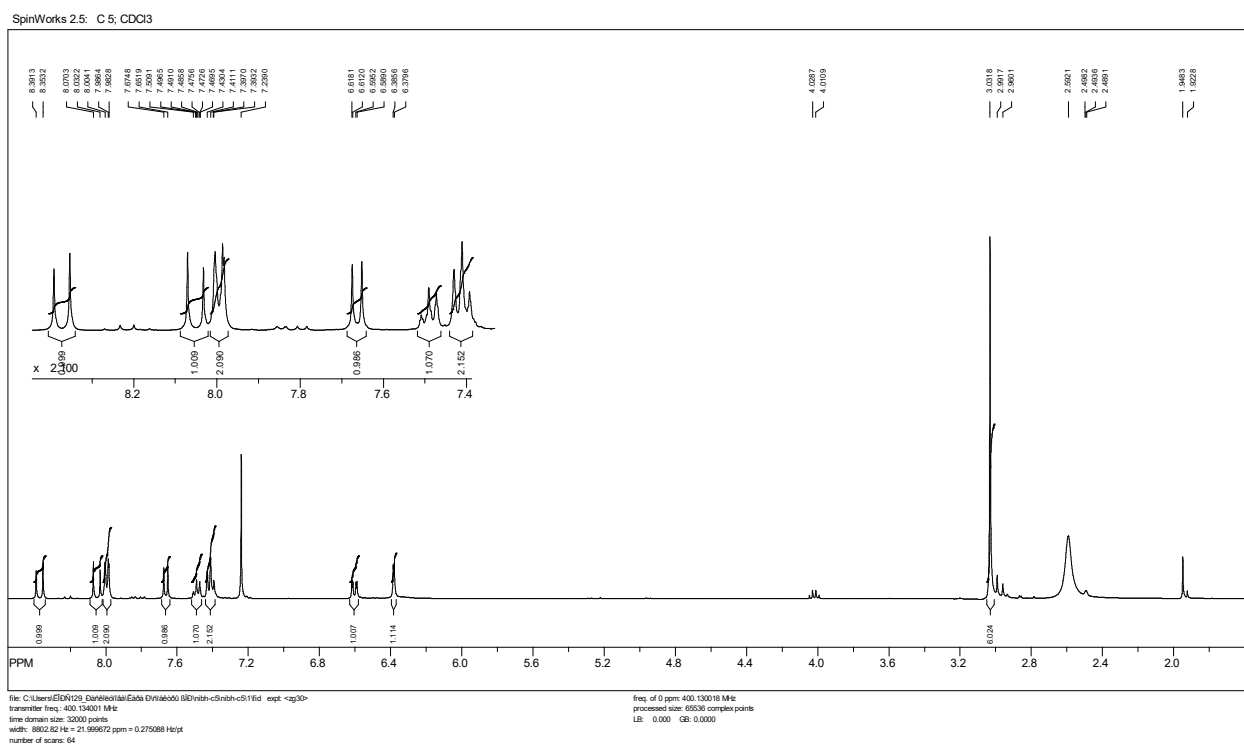
CN(C)c1ccc2c(c1)c(c(=O)c1ccccc1)c(Cl)c2=O<sup>1</sup>H NMR

**(E)-4-Chloro-7-(dimethylamino)-3-(3-oxo-3-phenylprop-1-en-1-yl)-2H-chromen-2-one (5)**

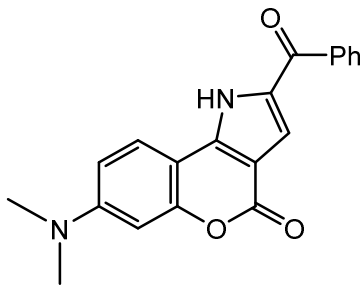


Coumarin (**4**) (1.32 g, 5.3 mmol) and (benzoylmethylene)triphenylphosphorane (2.02 g, 5.3 mmol,) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and stirred at RT for 24 h. The solvent was removed under reduced pressure and the residue was purified on a silica gel using gradient of EtOH in CH<sub>2</sub>Cl<sub>2</sub> (0-10%) as an eluent. The appropriate fractions were evaporated and then recrystallized from ethanol/acetonitrile (5/2) mixture to give orange crystals. Yield: 0.650 g, 1.8 mmol, 35%. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 8.37 (1H, d, *J* 15.2, CH=CH), 8.05 (1H, d, *J* 15.2, CH=CH), 8.01-7.97 (2H, m, *o*-Ph), 7.66 (1H, d, *J* 9.2, H5), 7.49 (1H, t, *J* 7.3, *p*-Ph), 7.41 (2H, t, *J* 7.4, *m*-Ph), 6.60 (1H, dd, *J* 9.1, 2.4, H6), 6.38 (1H, d, *J* 2.4, H8), 3.03 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>).

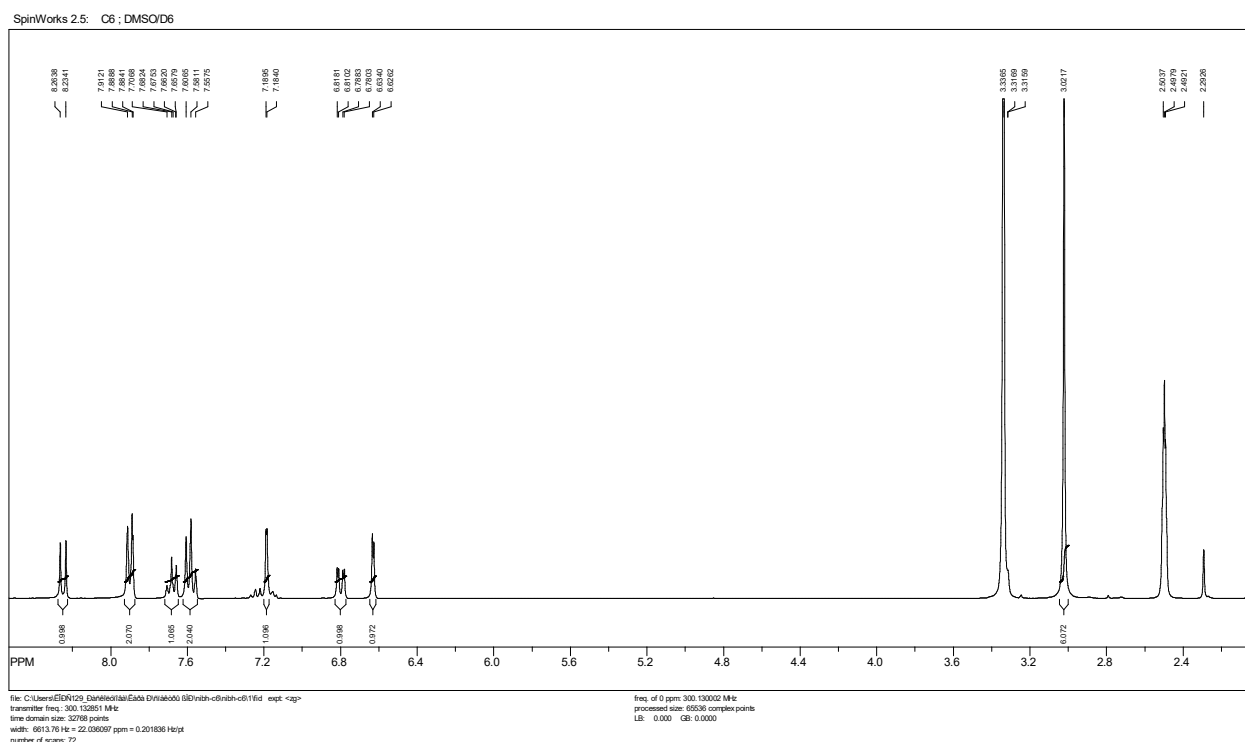
<sup>1</sup>H NMR



**2-Benzoyl-7-(dimethylamino)chromeno[4,3-b]pyrrol-4(1H)-one (7)**

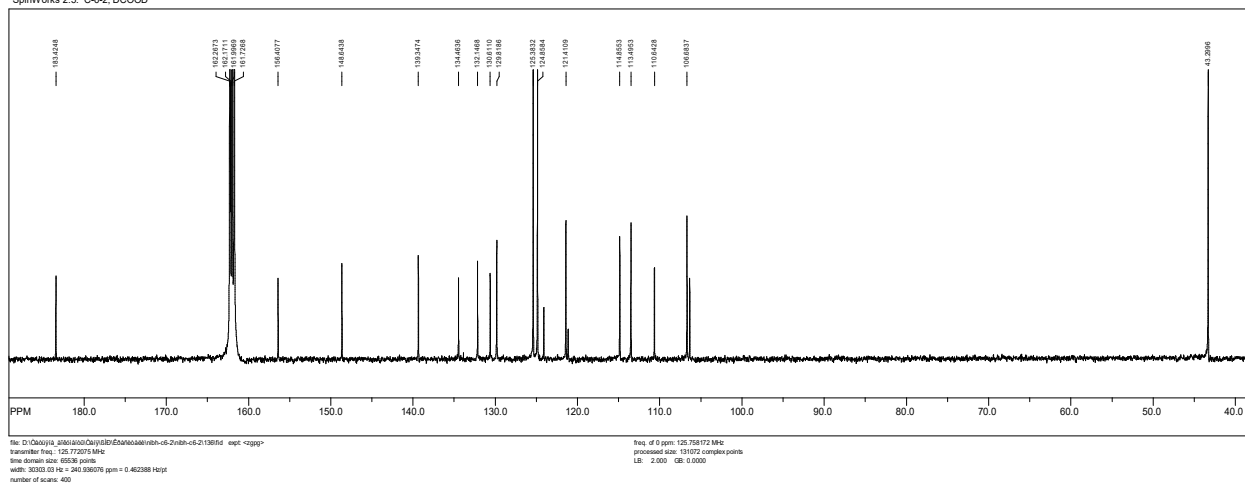


To a suspension of compound (**5**) (0.654 g, 1.9 mmol) in acetone (50 mL) sodium azide (0.200 g, 3.0 mmol) was added and the reaction mixture was vigorously stirred at 40°C for 2 h. Solvent was removed under reduced pressure and the residue was treated with water to give precipitate. Crystals were filtered off and dried in vacuum to give mixture of coumarins **6** and **7**. This solid mixture was suspended in dry toluene (15 mL) and refluxed for 2 h. Reaction mixture was cooled, the precipitate was filtered off, washed with toluene and dried to give pure title compound **7**. Light yellow powder. Yield: 0.554 g, 1.7 mmol, 88%. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 8.25 (1H, d, *J* 8.9, H9), 7.90 (2H, d, *J* 7.0, *o*-Ph), 7.68 (1H, t, *J* 7.4, *p*-Ph), 7.58 (2H, t, *J* 7.4, *m*-Ph), 7.19 (1H, d, *J* 1.6, H3), 6.80 (1H, dd, *J* 2.5, 9.1, H8), 6.63 (1H, d, *J* 2.4, H6), 3.02 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR (D<sub>2</sub>O): 183.43, 162.17, 156.41, 148.64, 139.35, 134.46, 132.15, 130.62, 129.83, 125.39, 124.86, 121.41, 114.86, 113.50, 110.65, 106.69, 43.29; MS ESI (*m/z*): [M+H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>17</sub>N<sub>2</sub>O<sub>3</sub> 333.12; found 333.00.

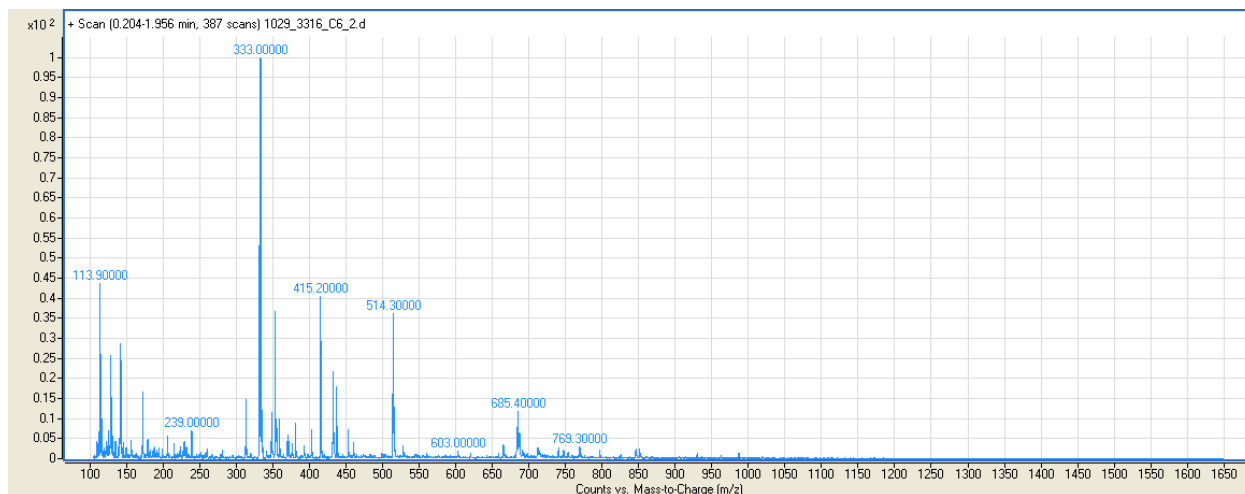
<sup>1</sup>H NMR

<sup>13</sup>C NMR

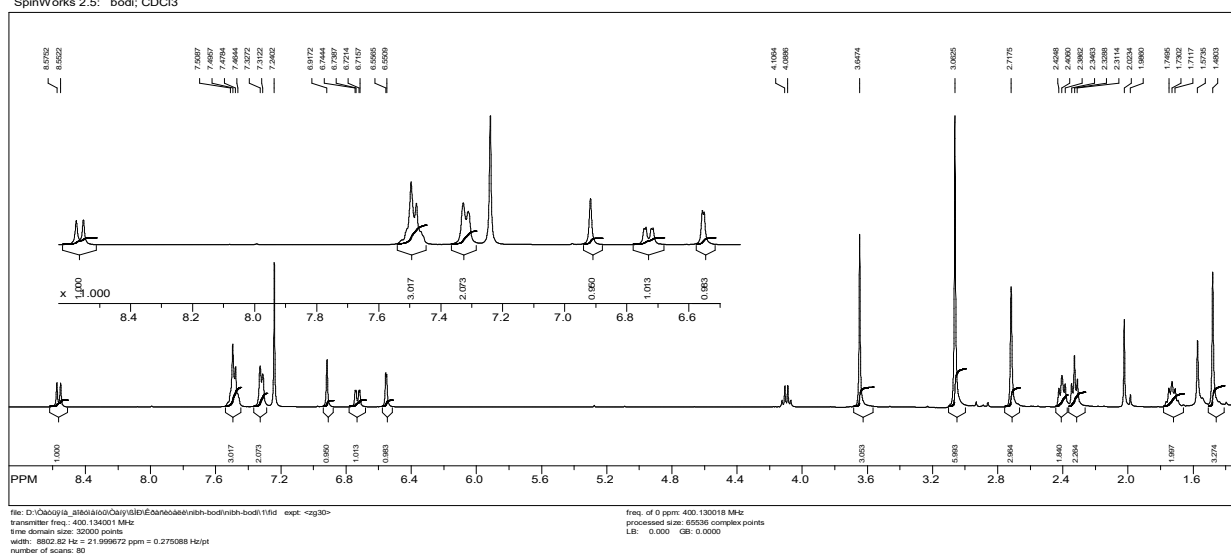
SpinWorks 2.5: C-6-2; DCOOD



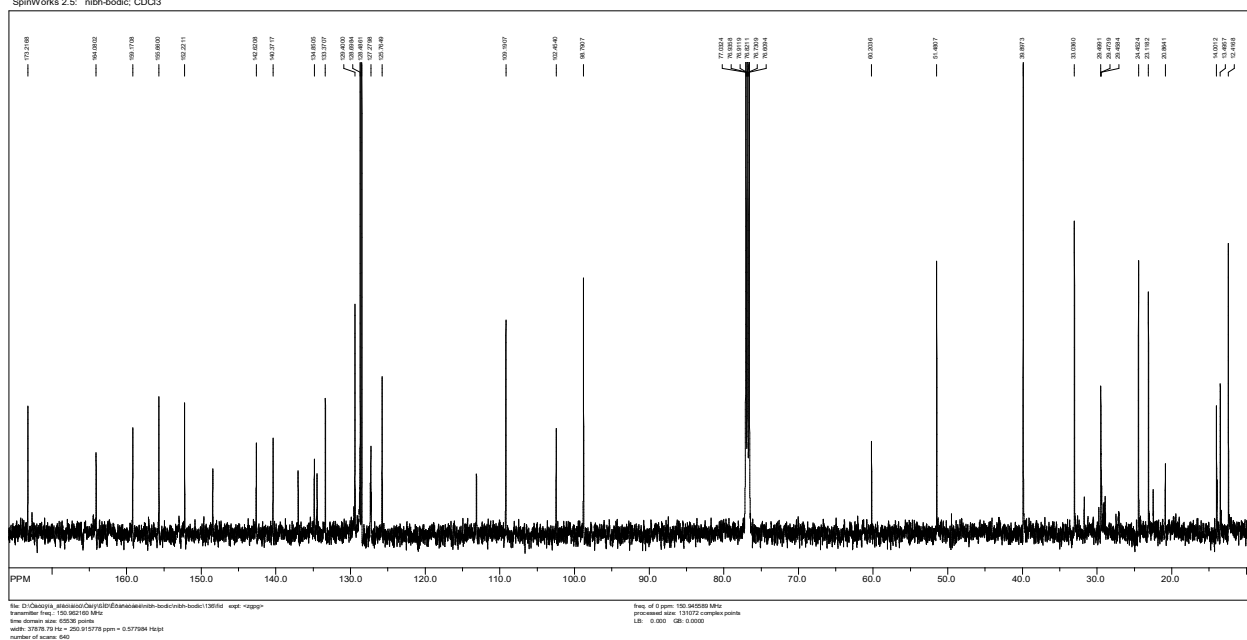
## ESI mass spectra



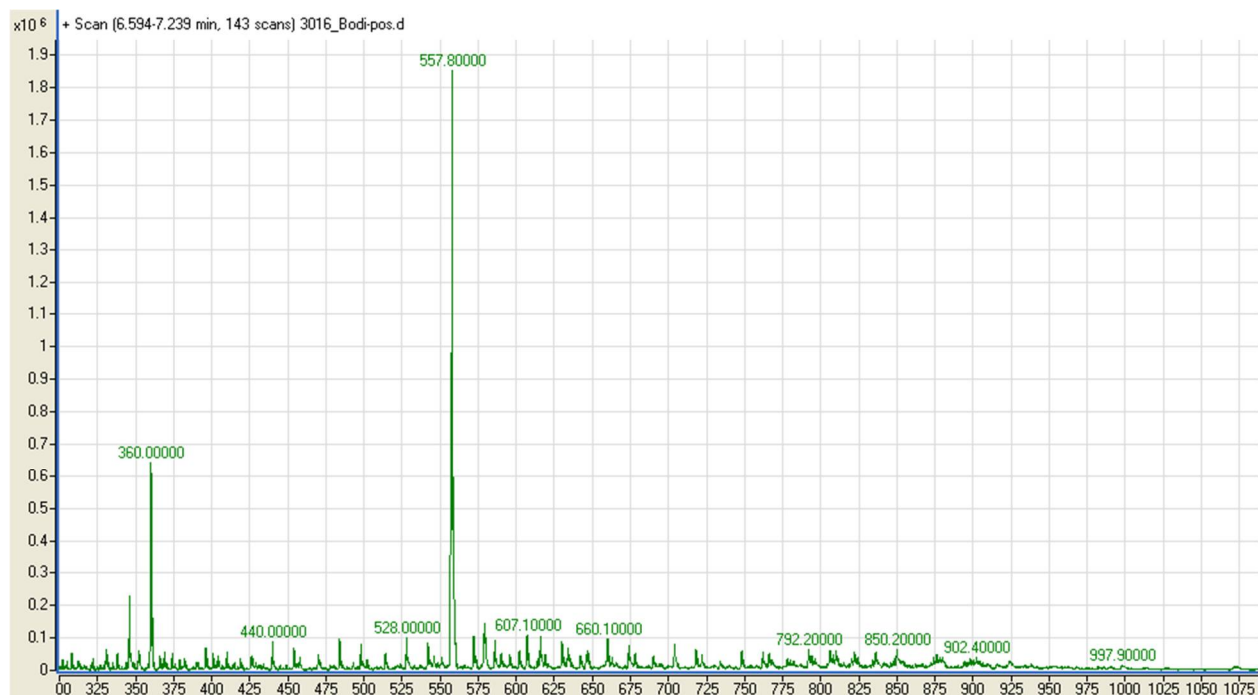


SpinWorks 2.5: bodi; CDCl<sub>3</sub>

SpinWorks 2.5: nibh-bodic; CDCl<sub>3</sub>

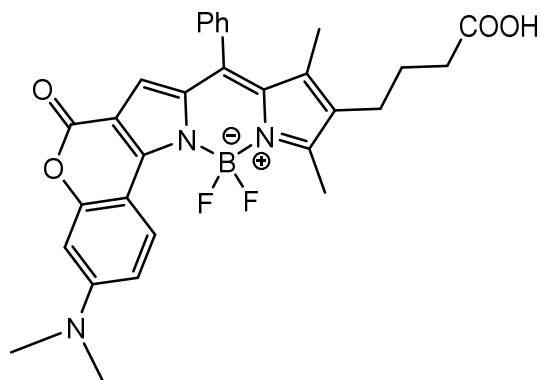


## 5

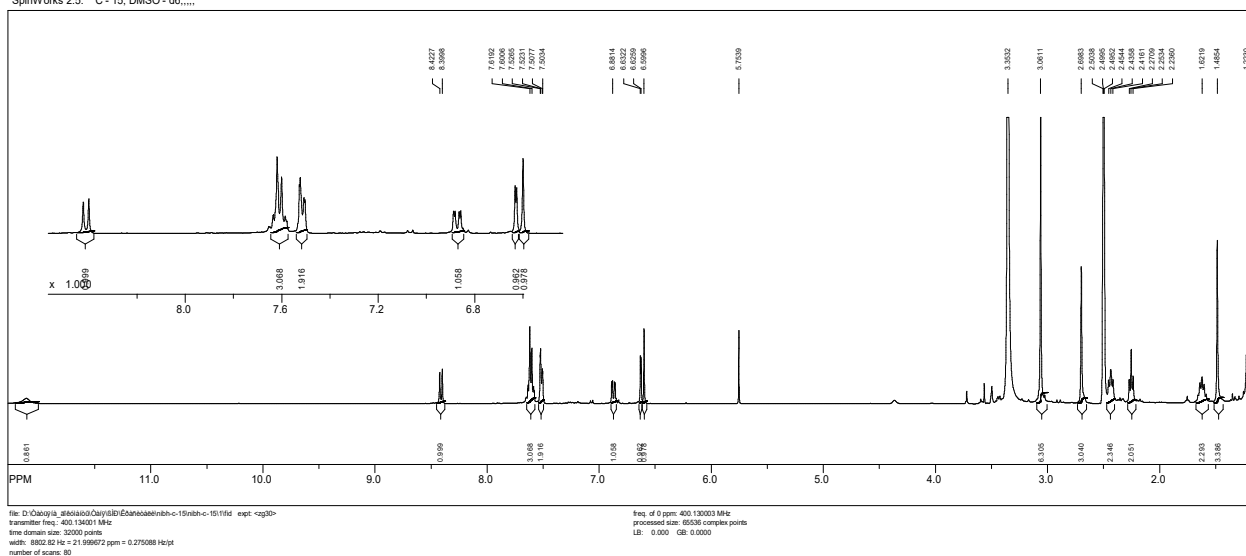




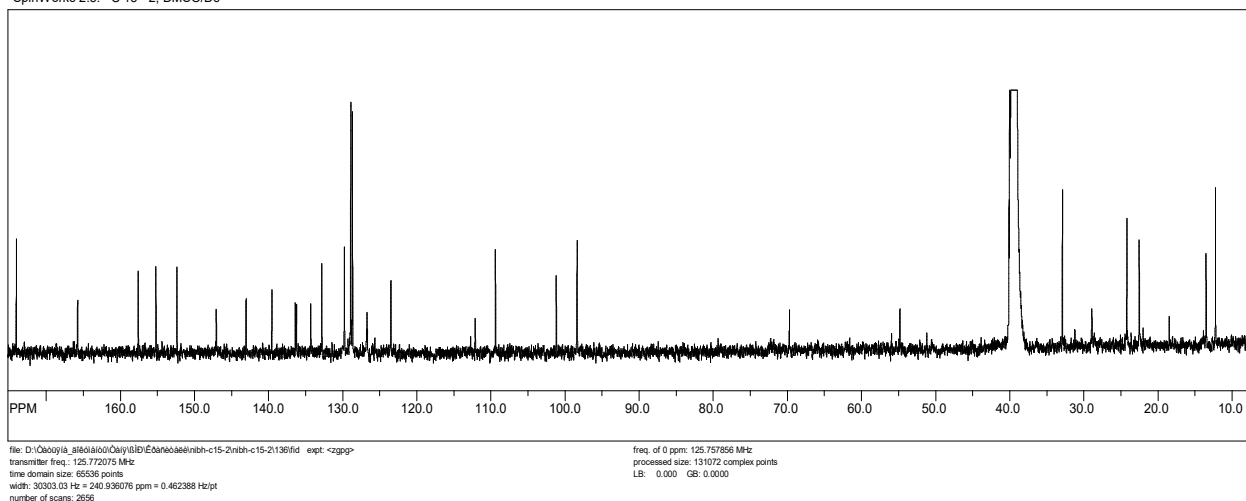
### BODIPY-COOH (1)

<sup>1</sup>H NMR

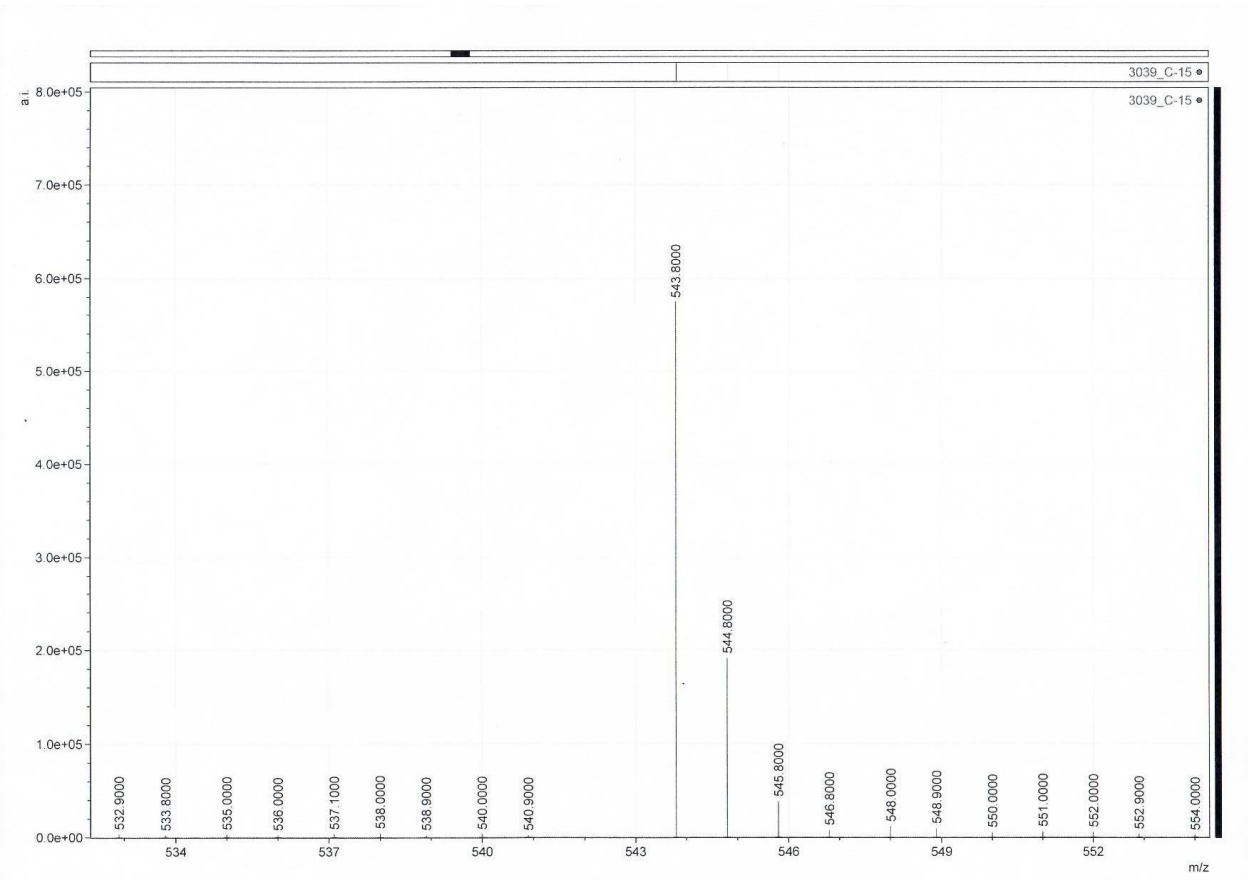
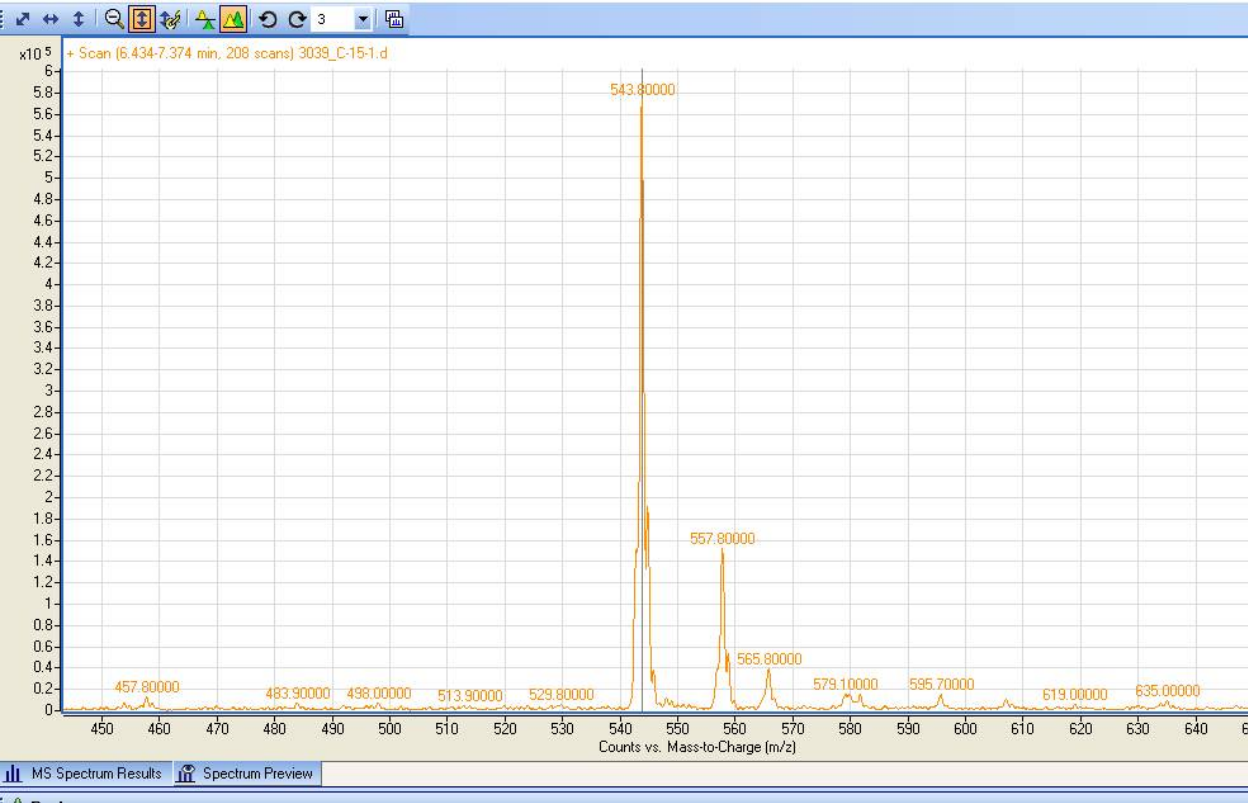
SpinWorks 2.5: C - 15; DMSO - d6;

<sup>13</sup>C NMR

SpinWorks 2.5: C 15 - 2; DMSO/D6

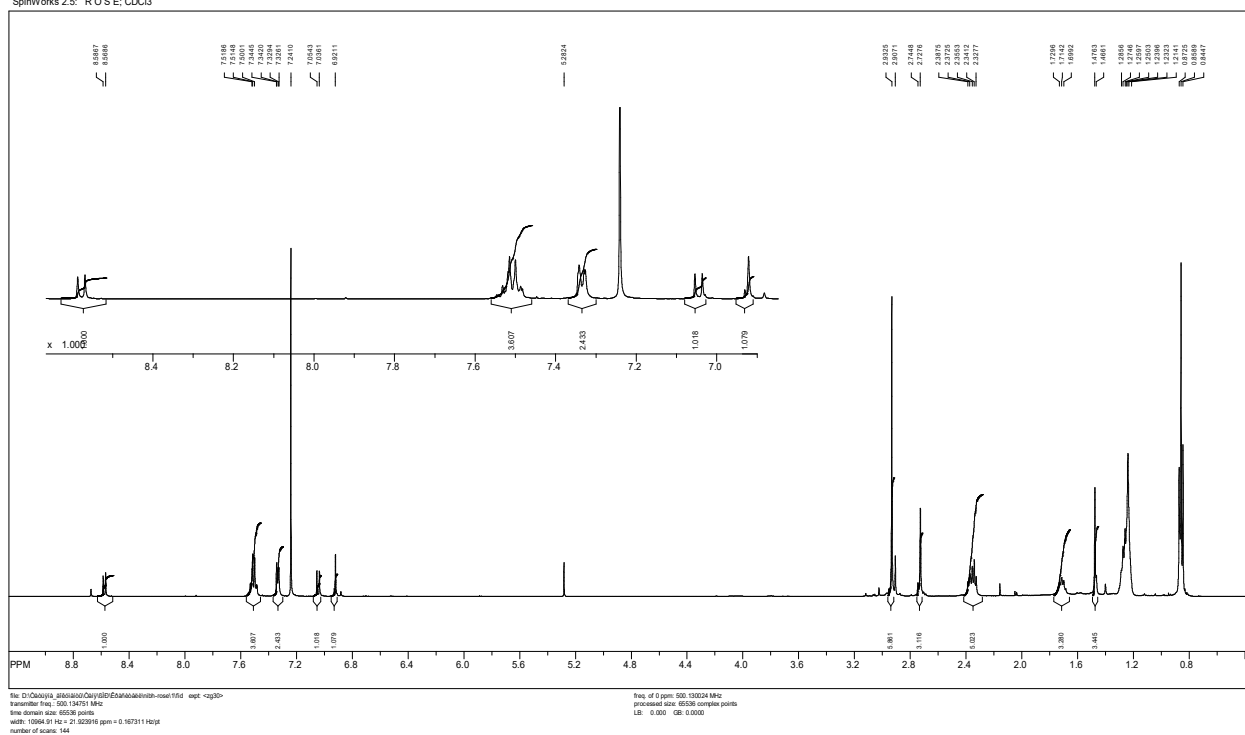
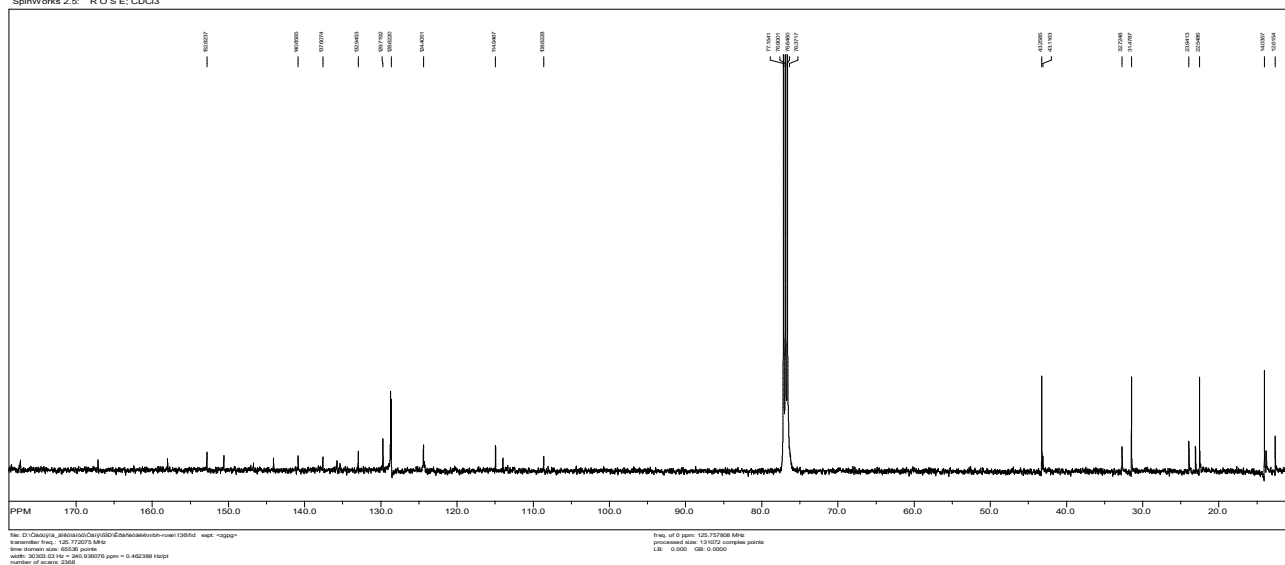


ESI mass spectra

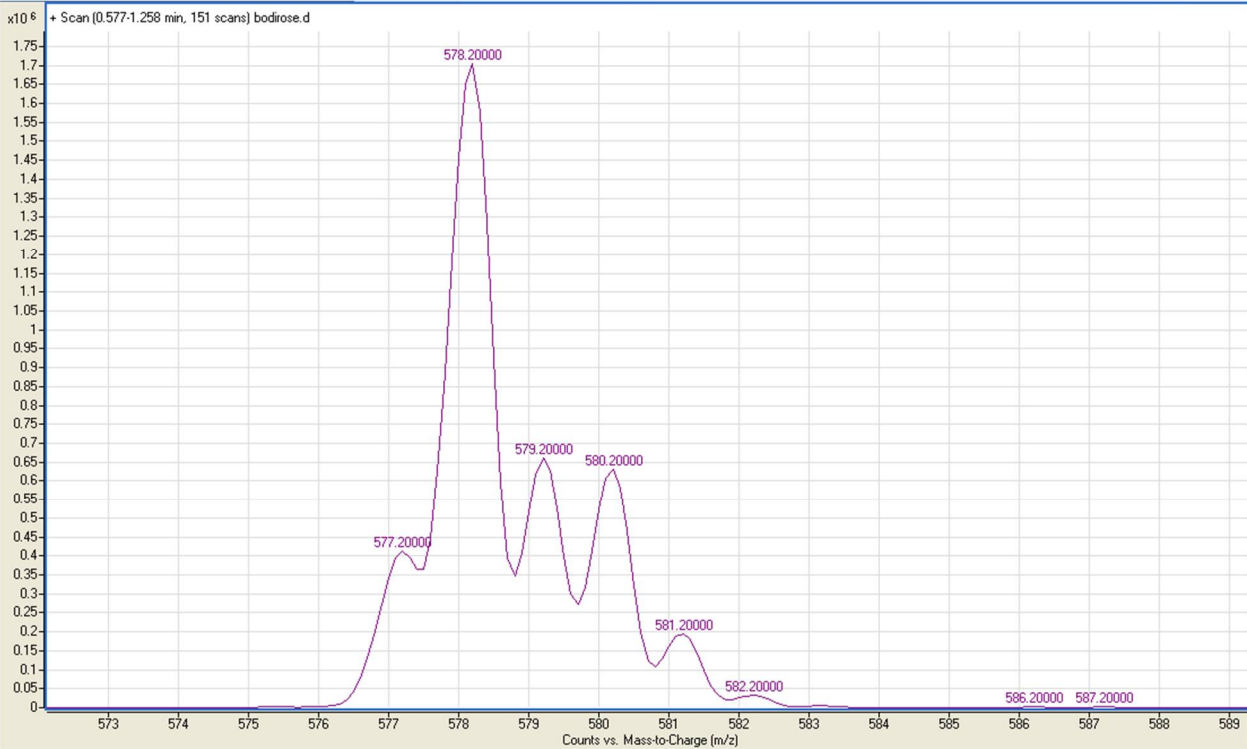
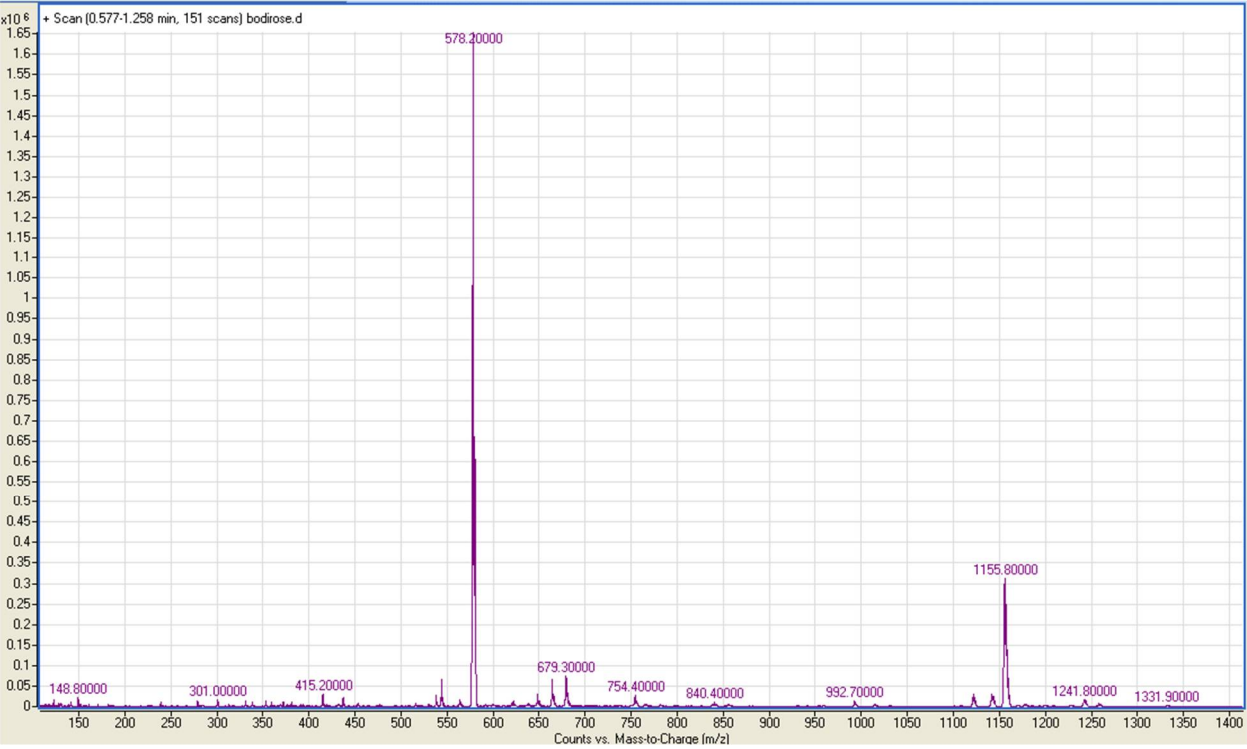


CN(C)c1ccc2c(c1)Oc3cc4c(cc2C3=CC=C4)N(C5=CC=C(C=C5)C(=C6C(=CC(=C6)C(=C7C(=CC(=C7)C(=C8C(=CC(=C8)C(=C9C(=CC(=C9)C(=C10C(=CC(=C10)C(=C11C(=CC(=C11)C(=C12C(=CC(=C12)C(=C13C(=CC(=C13)C(=C14C(=CC(=C14)C(=C15C(=CC(=C15)C(=C16C(=CC(=C16)C(=C17C(=CC(=C17)C(=C18C(=CC(=C18)C(=C19C(=CC(=C19)C(=C20C(=CC(=C20)C(=C21C(=CC(=C21)C(=C22C(=CC(=C22)C(=C23C(=CC(=C23)C(=C24C(=CC(=C24)C(=C25C(=CC(=C25)C(=C26C(=CC(=C26)C(=C27C(=CC(=C27)C(=C28C(=CC(=C28)C(=C29C(=CC(=C29)C(=C30C(=CC(=C30)C(=C31C(=CC(=C31)C(=C32C(=CC(=C32)C(=C33C(=CC(=C33)C(=C34C(=CC(=C34)C(=C35C(=CC(=C35)C(=C36C(=CC(=C36)C(=C37C(=CC(=C37)C(=C38C(=CC(=C38)C(=C39C(=CC(=C39)C(=C40C(=CC(=C40)C(=C41C(=CC(=C41)C(=C42C(=CC(=C42)C(=C43C(=CC(=C43)C(=C44C(=CC(=C44)C(=C45C(=CC(=C45)C(=C46C(=CC(=C46)C(=C47C(=CC(=C47)C(=C48C(=CC(=C48)C(=C49C(=CC(=C49)C(=C50C(=CC(=C50)C(=C51C(=CC(=C51)C(=C52C(=CC(=C52)C(=C53C(=CC(=C53)C(=C54C(=CC(=C54)C(=C55C(=CC(=C55)C(=C56C(=CC(=C56)C(=C57C(=CC(=C57)C(=C58C(=CC(=C58)C(=C59C(=CC(=C59)C(=C60C(=CC(=C60)C(=C61C(=CC(=C61)C(=C62C(=CC(=C62)C(=C63C(=CC(=C63)C(=C64C(=CC(=C64)C(=C65C(=CC(=C65)C(=C66C(=CC(=C66)C(=C67C(=CC(=C67)C(=C68C(=CC(=C68)C(=C69C(=CC(=C69)C(=C70C(=CC(=C70)C(=C71C(=CC(=C71)C(=C72C(=CC(=C72)C(=C73C(=CC(=C73)C(=C74C(=CC(=C74)C(=C75C(=CC(=C75)C(=C76C(=CC(=C76)C(=C77C(=CC(=C77)C(=C78C(=CC(=C78)C(=C79C(=CC(=C79)C(=C80C(=CC(=C80)C(=C81C(=CC(=C81)C(=C82C(=CC(=C82)C(=C83C(=CC(=C83)C(=C84C(=CC(=C84)C(=C85C(=CC(=C85)C(=C86C(=CC(=C86)C(=C87C(=CC(=C87)C(=C88C(=CC(=C88)C(=C89C(=CC(=C89)C(=C90C(=CC(=C90)C(=C91C(=CC(=C91)C(=C92C(=CC(=C92)C(=C93C(=CC(=C93)C(=C94C(=CC(=C94)C(=C95C(=CC(=C95)C(=C96C(=CC(=C96)C(=C97C(=CC(=C97)C(=C98C(=CC(=C98)C(=C99C(=CC(=C99)C(=C100C(=CC(=C100)C(=C101C(=CC(=C101)C(=C102C(=CC(=C102)C(=C103C(=CC(=C103)C(=C104C(=CC(=C104)C(=C105C(=CC(=C105)C(=C106C(=CC(=C106)C(=C107C(=CC(=C107)C(=C108C(=CC(=C108)C(=C109C(=CC(=C109)C(=C110C(=CC(=C110)C(=C111C(=CC(=C111)C(=C112C(=CC(=C112)C(=C113C(=CC(=C113)C(=C114C(=CC(=C114)C(=C115C(=CC(=C115)C(=C116C(=CC(=C116)C(=C117C(=CC(=C117)C(=C118C(=CC(=C118)C(=C119C(=CC(=C119)C(=C120C(=CC(=C120)C(=C121C(=CC(=C121)C(=C122C(=CC(=C122)C(=C123C(=CC(=C123)C(=C124C(=CC(=C124)C(=C125C(=CC(=C125)C(=C126C(=CC(=C126)C(=C127C(=CC(=C127)C(=C128C(=CC(=C128)C(=C129C(=CC(=C129)C(=C130C(=CC(=C130)C(=C131C(=CC(=C131)C(=C132C(=CC(=C132)C(=C133C(=CC(=C133)C(=C134C(=CC(=C134)C(=C135C(=CC(=C135)C(=C136C(=CC(=C136)C(=C137C(=CC(=C137)C(=C138C(=CC(=C138)C(=C139C(=CC(=C139)C(=C140C(=CC(=C140)C(=C141C(=CC(=C141)C(=C142C(=CC(=C142)C(=C143C(=CC(=C143)C(=C144C(=CC(=C144)C(=C145C(=CC(=C145)C(=C146C(=CC(=C146)C(=C147C(=CC(=C147)C(=C148C(=CC(=C148)C(=C149C(=CC(=C149)C(=C150C(=CC(=C150)C(=C151C(=CC(=C151)C(=C152C(=CC(=C152)C(=C153C(=CC(=C153)C(=C154C(=CC(=C154)C(=C155C(=CC(=C155)C(=C156C(=CC(=C156)C(=C157C(=CC(=C157)C(=C158C(=CC(=C158)C(=C159C(=CC(=C159)C(=C160C(=CC(=C160)C(=C161C(=CC(=C161)C(=C162C(=CC(=C162)C(=C163C(=CC(=C163)C(=C164C(=CC(=C164)C(=C165C(=CC(=C165)C(=C166C(=CC(=C166)C(=C167C(=CC(=C167)C(=C168C(=CC(=C168)C(=C169C(=CC(=C169)C(=C170C(=CC(=C170)C(=C171C(=CC(=C171)C(=C172C(=CC(=C172)C(=C173C(=CC(=C173)C(=C174C(=CC(=C174)C(=C175C(=CC(=C175)C(=C176C(=CC(=C176)C(=C177C(=CC(=C177)C(=C178C(=CC(=C178)C(=C179C(=CC(=C179)C(=C180C(=CC(=C180)C(=C181C(=CC(=C181)C(=C182C(=CC(=C182)C(=C183C(=CC(=C183)C(=C184C(=CC(=C184)C(=C185C(=CC(=C185)C(=C186C(=CC(=C186)C(=C187C(=CC(=C187)C(=C188C(=CC(=C188)C(=C189C(=CC(=C189)C(=C190C(=CC(=C190)C(=C191C(=CC(=C191)C(=C192C(=CC(=C192)C(=C193C(=CC(=C193)C(=C194C(=CC(=C194)C(=C195C(=CC(=C195)C(=C196C(=CC(=C196)C(=C197C(=CC(=C197)C(=C198C(=CC(=C198)C(=C199C(=CC(=C199)C(=C200C(=CC(=C200)C(=C201C(=CC(=C201)C(=C202C(=CC(=C202)C(=C203C(=CC(=C203)C(=C204C(=CC(=C204)C(=C205C(=CC(=C205)C(=C206C(=CC(=C206)C(=C207C(=CC(=C207)C(=C208C(=CC(=C208)C(=C209C(=CC(=C209)C(=C210C(=CC(=C210)C(=C211C(=CC(=C211)C(=C212C(=CC(=C212)C(=C213C(=CC(=C213)C(=C214C(=CC(=C214)C(=C215C(=CC(=C215)C(=C216C(=CC(=C216)C(=C217C(=CC(=C217)C(=C218C(=CC(=C218)C(=C219C(=CC(=C219)C(=C220C(=CC(=C220)C(=C221C(=CC(=C221)C(=C222C(=CC(=C222)C(=C223C(=CC(=C223)C(=C224C(=CC(=C224)C(=C225C(=CC(=C225)C(=C226C(=CC(=C226)C(=C227C(=CC(=C227)C(=C228C(=CC(=C228)C(=C229C(=CC(=C229)C(=C230C(=CC(=C230)C(=C231C(=CC(=C231)C(=C232C(=CC(=C232)C(=C233C(=CC(=C233)C(=C234C(=CC(=C234)C(=C235C(=CC(=C235)C(=C236C(=CC(=C236)C(=C237C(=CC(=C237)C(=C238C(=CC(=C238)C(=C239C(=CC(=C239)C(=C240C(=CC(=C240)C(=C241C(=CC(=C241)C(=C242

SpinWorks 2.5: R O S E; CDCl<sub>3</sub>

SpinWorks 2.5: R O S E; CDCl<sub>3</sub>

ESI mass spectra



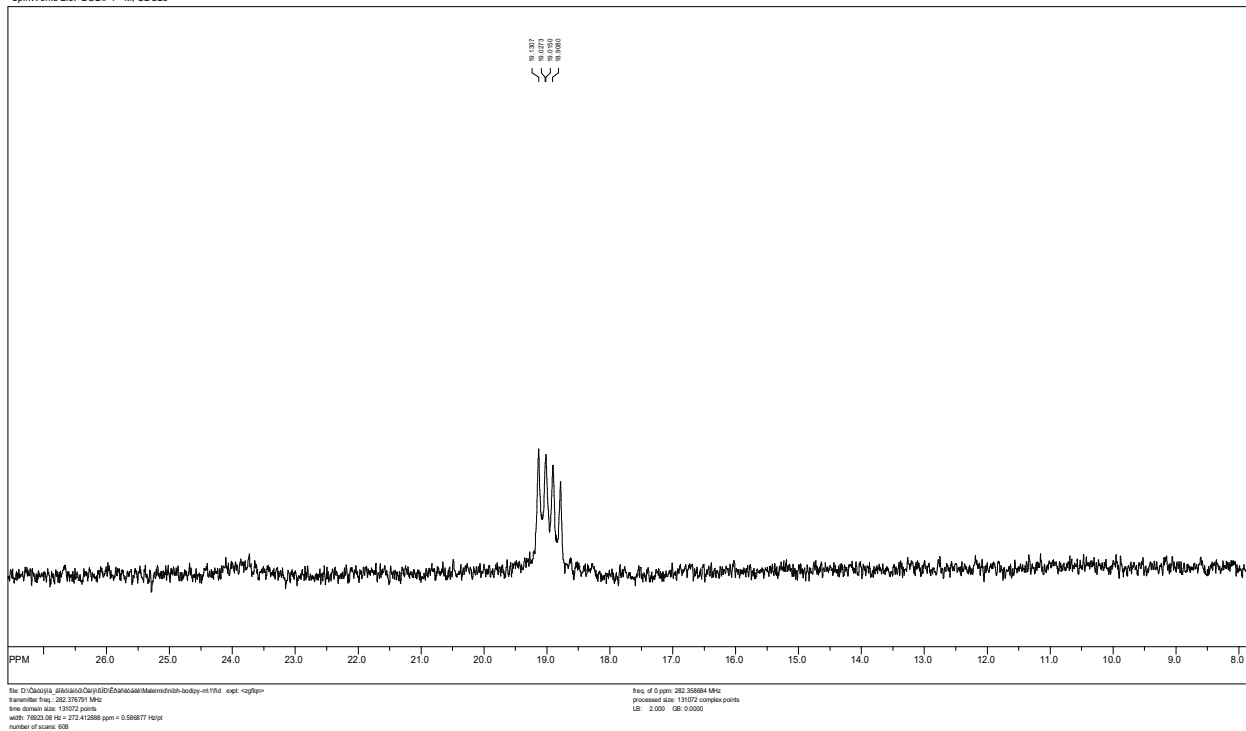
Chemical structure of compound 10, a fluoroborate derivative. The structure shows a central boron atom (B) with a positive charge, coordinated by two fluorine atoms (F) and two nitrogen atoms (N). One nitrogen is part of a complex fused ring system including a benzene ring and a lactone. The other nitrogen is part of a pyrrole ring substituted with a phenyl group (Ph) and a long chain ending in a pyrrolidinone ring.

## SpoinWorks 2.5: BODIM: CDCL3

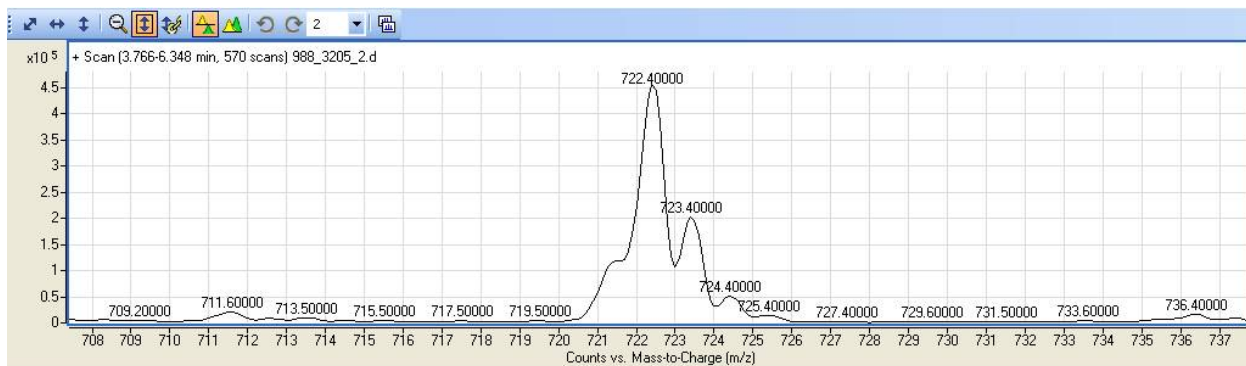
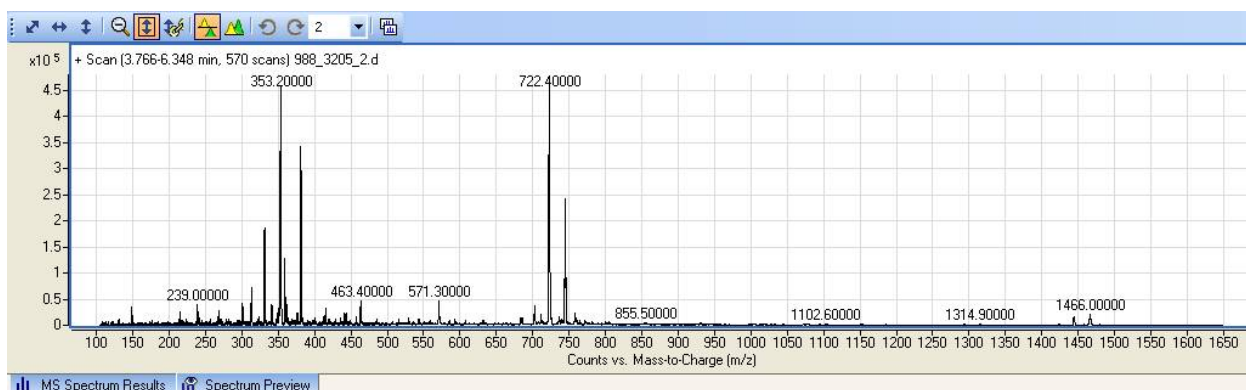


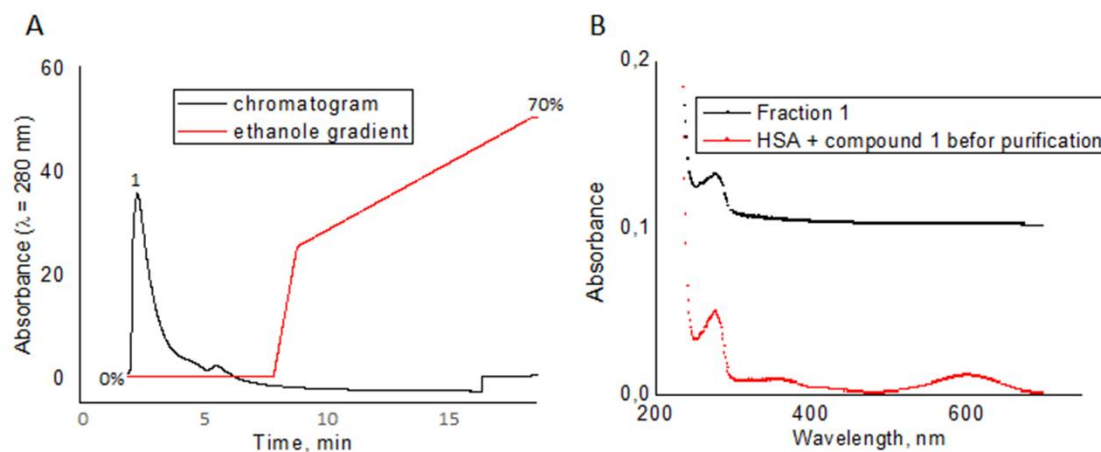
<sup>19</sup>F NMR

SpinWorks 2.5: BODIPY - M; CDCL3



## ESI mass spectra





**Figure S1.** Panel A: ion exchange HPLC of a mixture of HSA and BODIPY dye (**1**). Polysil CA-500 column, 10  $\mu$ m, 250 x 4.6 mm., elution: 0.3 M phosphate buffer, pH 7.4, ethanol gradient 0-70%, UV detection at 280 nm; panel B: UV-vis spectra of the HSA and BODIPY (**1**) mixture (red line) and fraction 1 (black line).