Electronic Supplementary Material (ESI) for Analyst.

supporting information

Development of highly sensitive fluorescent probes for the detection of β-galactosidase activity – application to the real-time monitoring of senescence in live cells.

Mauro Safir Filho,[a,b,†] Pascal Dao,[a,†]Maeva Gesson,[c] Anthony R. Martin,[a,*]Rachid Benhida[a,*]

[a] Université Côte d'Azur, CNRS, Institut de Chimie de Nice UMR 7272, Nice, France

[b] CAPES Foundation, Ministry of Education of Brazil, Brasília DF 70040-020, Brazil

[c]Université Côte d'Azur, Centre Méditerranéen de Médecine Moléculaire (C3M), INSERM U1065, Nice, France

* corresponding authors: *anmartin@unice.fr*, *benhida@unice.fr*.

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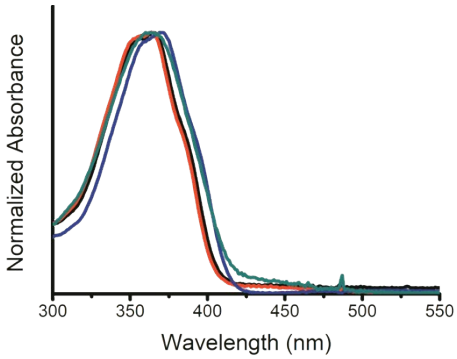
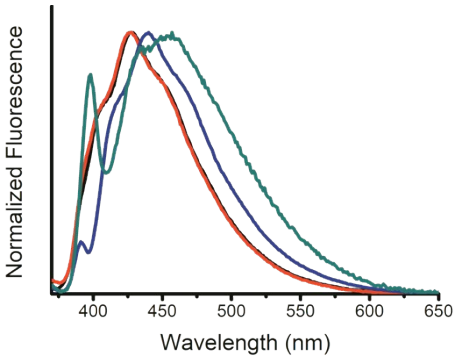
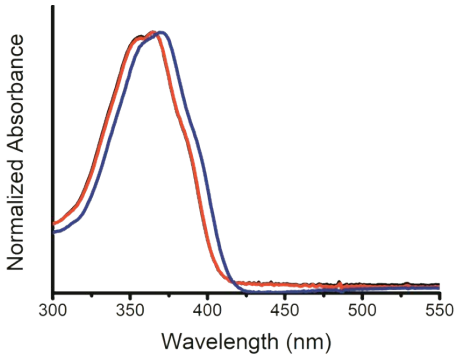
Photophysical Studies

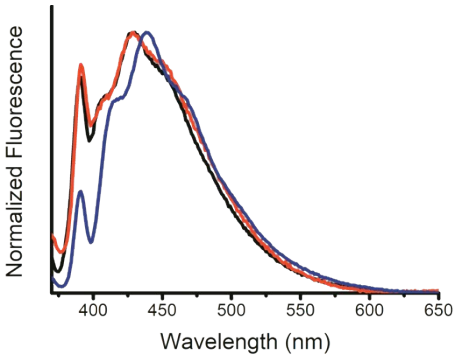
βGal-1





βGal-2





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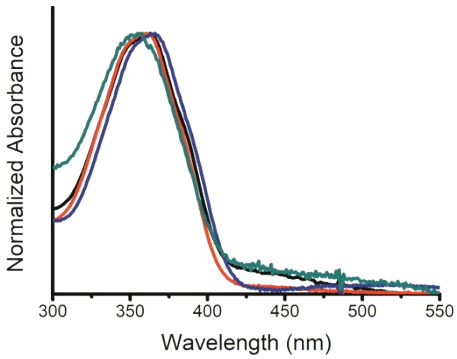
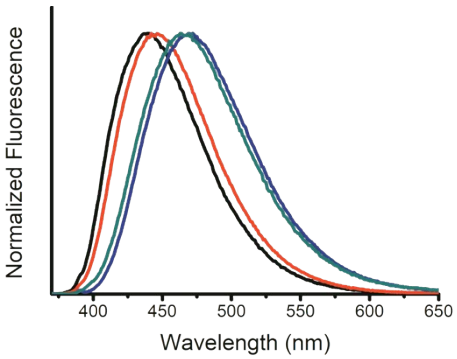
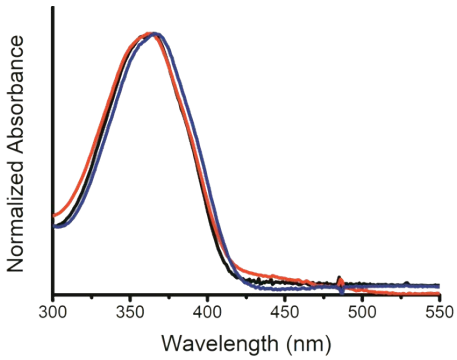
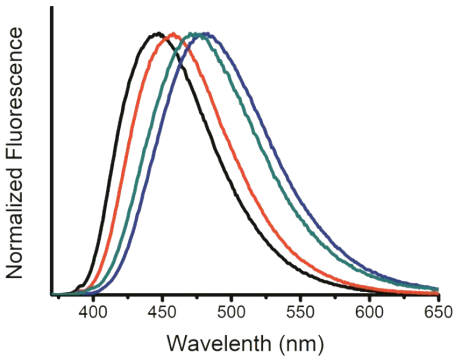




βGal-4





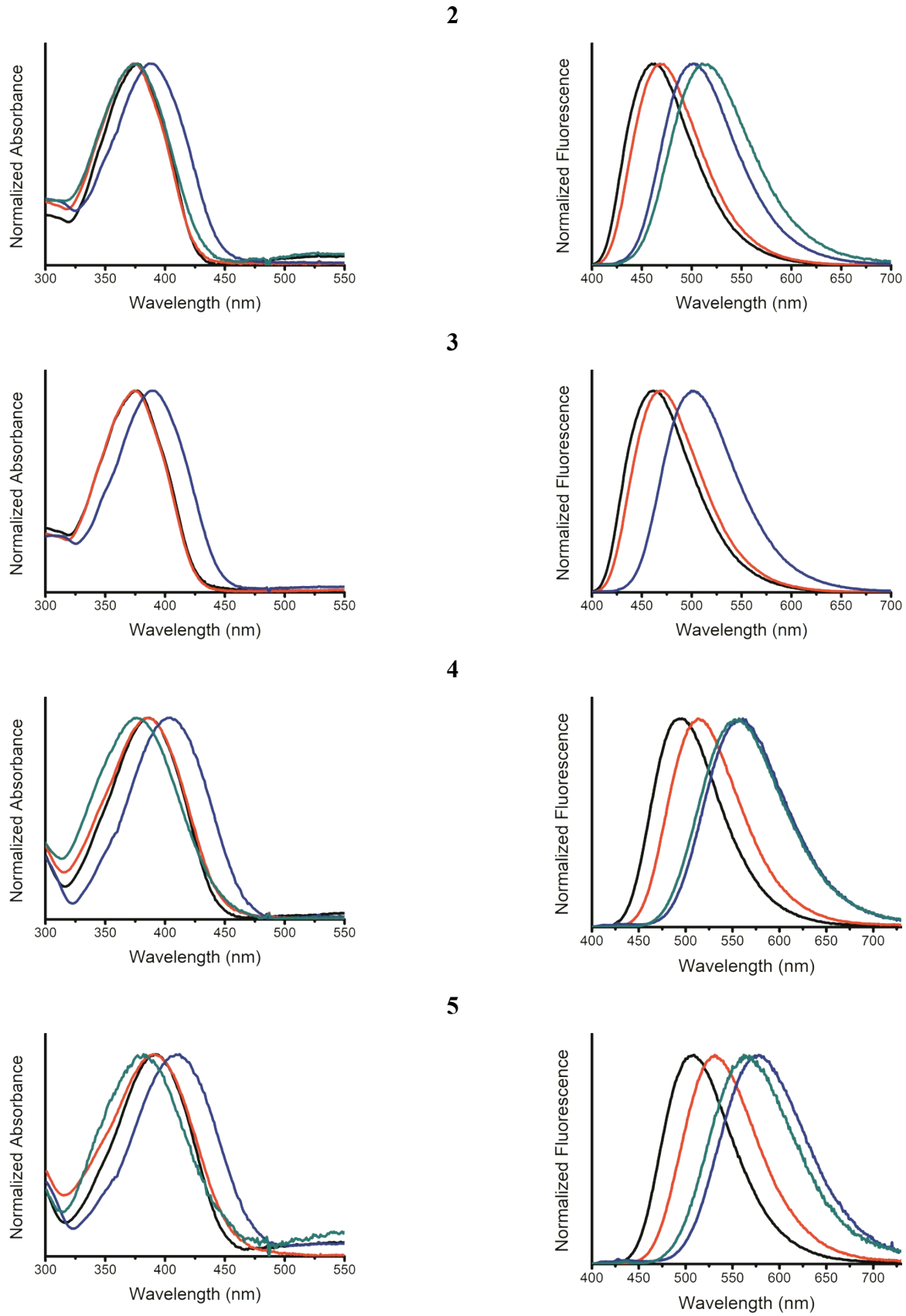


Fig S1. Normalized absorption and fluorescence emission spectra of probes **βGal-1-4** and the respective fluorescent reporter dyes **2-5** in 1,4-dioxane (black), ethyl acetate (red), DMSO (blue) and PBS/DMSO (8/2) (green).

Table S1. Main photophysical data from the UV-visible absorption and steady-state fluorescence emission of dyes **2-5** and probes **βGal-1-4**.

Compound	Solvent	λ_{abs} (nm)	λ_{em} (nm)	$\Delta\lambda$ (nm/cm^{-1})	ε x 10^3 (M.cm)$^{-1}$	Φ_{fl}[a]
2	1,4-dioxane	379	463	84/4786	33.0	0.45
	Ethyl acetate	374	466	92/5278	34.2	0.34
	DMSO	388	501	113/5813	31.0	0.52
	PBS	376	514	138/7140	29.4	0.32
3	1-4,dioxane	375	462	87/5021	37.0	0.59
	Ethyl acetate	375	470	95/5390	38.2	0.57
	DMSO	389	502	113/5786	35.4	0.61
	PBS	-	501	-	-	-
4	1-4,dioxane	385	494	109/5731	27.3	0.60
	Ethyl acetate	385	513	128/6480	28.5	0.52
	DMSO	403	560	157/6956	23.0	0.58
	PBS	376	555	179/8577	26.2	0.30
5	1-4,dioxane	391	508	117/5890	24.2	0.52
	Ethyl acetate	391	531	140/6743	26.7	0.45
	DMSO	410	577	167/7059	21.0	0.41
	PBS	380	565	185/8616	20.2	0.14
βgal-1	1-4,dioxane	361	427	66/4281	36.2	<0.005
	Ethyl acetate	362	426	64/4150	37.1	<0.005
	DMSO	370	439	69/4247	34.8	<0.005
	PBS	364	455	91/5494	33.4	<0.005
βgal-2	1-4,dioxane	363	428	65/4183	37.3	<0.005
	Ethyl acetate	363	428	65/4183	38.1	<0.005
	DMSO	370	439	69/4247	34.7	<0.005
	PBS	-	473	-	-	-
βgal-3	1-4,dioxane	361	440	79/4973	33.5	0.31
	Ethyl acetate	361	445	84/5228	30.2	0.41
	DMSO	366	469	103/6000	28.2	0.47
	PBS	354	466	112/6789	30.5	0.21
βgal-4	1-4,dioxane	363	440	77/4820	29.9	0.38
	Ethyl acetate	363	458	95/5714	33.2	0.39
	DMSO	366	480	114/6489	28.3	0.41
	PBS	-	475	-	-	-

a - Fluorescence quantum yields were measured using quinine sulfate in aqueous 0.1M $HClO_4$ solution (Φ_{fl} = 0.54, λ_{exc} = 350 nm) as reference.

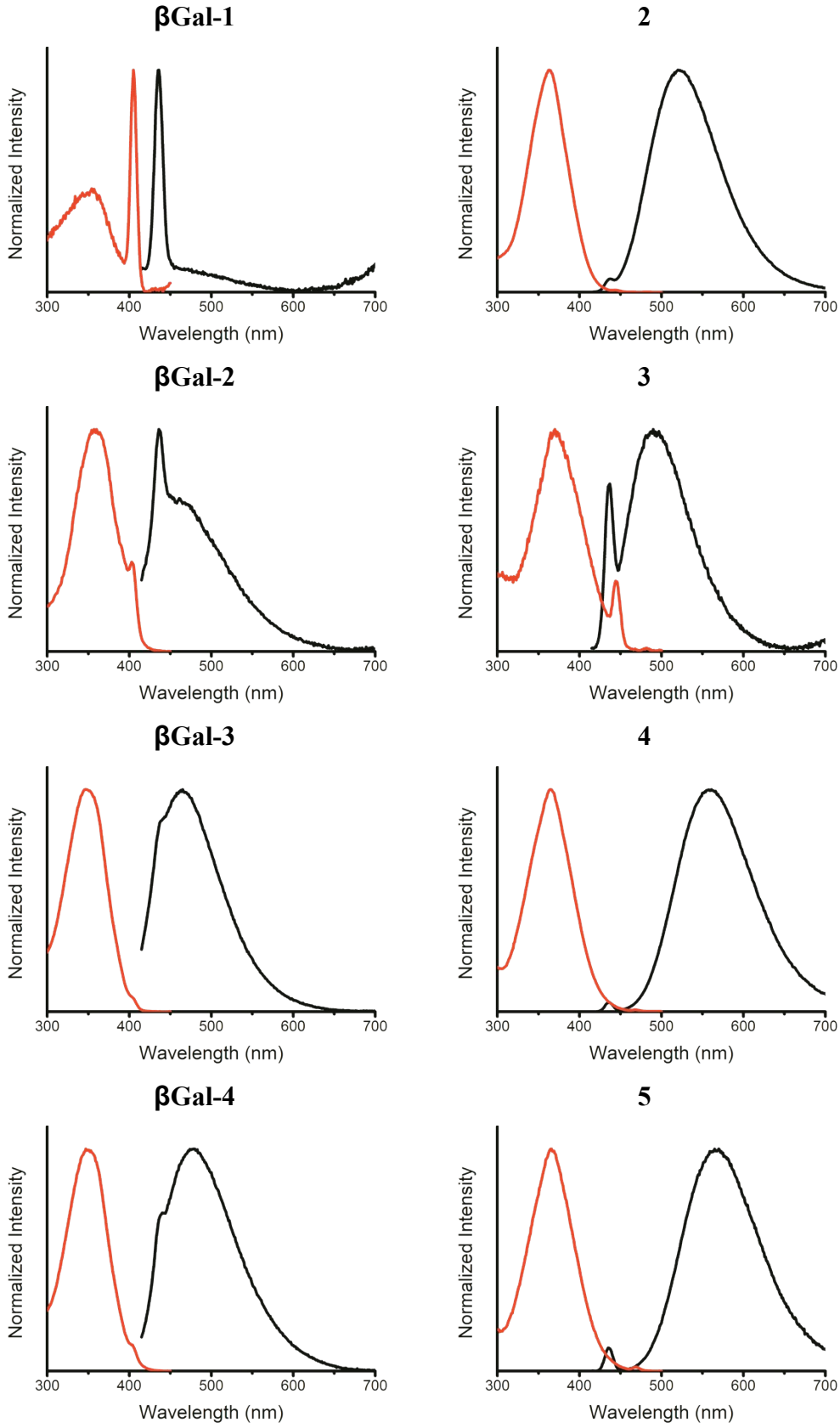


Fig S2. Normalized excitation (red) and emission (black) spectra of probes **βGal-1**, **3** and **4** and the respective fluorescent reporter dyes **2,4** and **5** in PBS (pH 7.4).

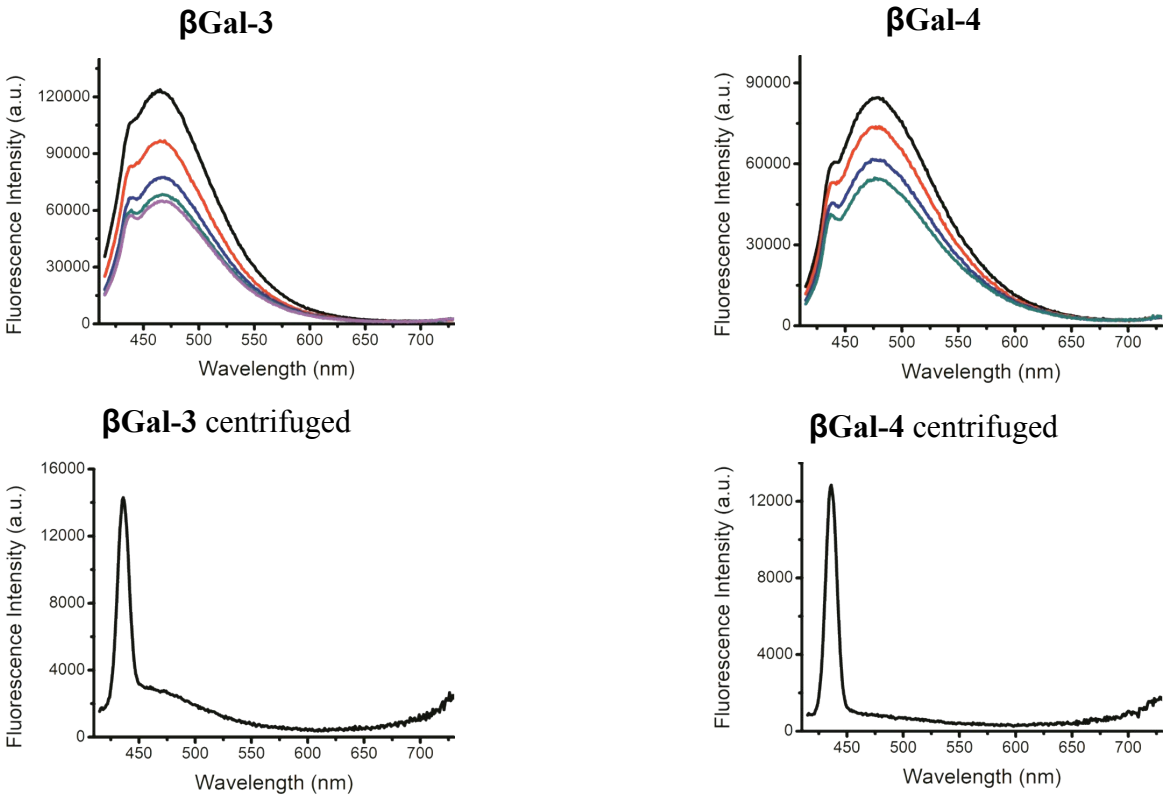


Fig S3. Time-dependent fluorescence emission spectra of probes **βGal-3** and **βGal-4** (0.2 μM, PBS pH 7.4, 0-5 min) (up). Respective fluorescent emission spectra of the corresponding centrifuged solutions of **βGal-3** and **βGal-4** (down).

<u>βGal response</u>

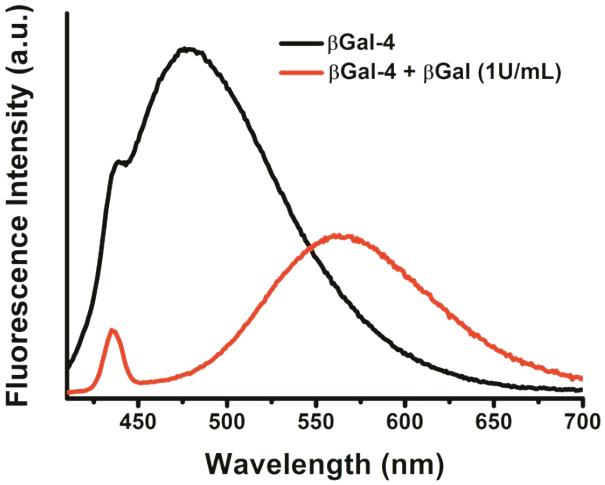
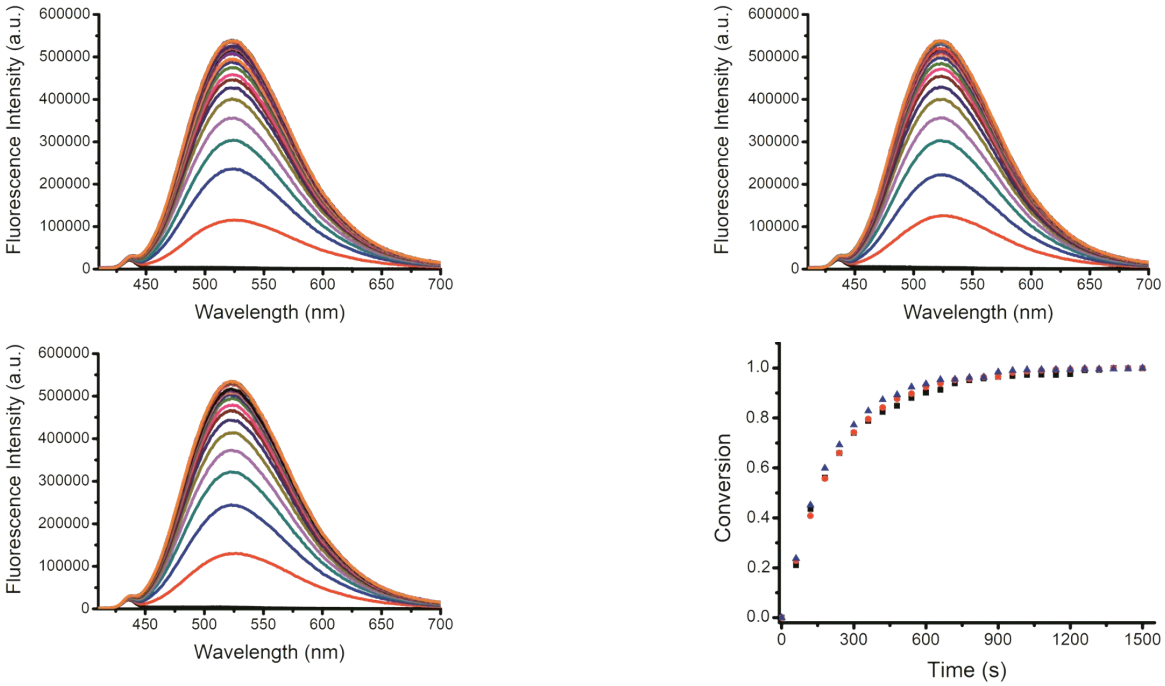


Fig S4. Fluorescence emission spectra of **βGal-4** in absence (black) and after treatment with 1 U/mL of β-Gal (red) after 10 minutes. Conditions: dye concentration ≈ 0.2 μM; λ_{exc} = 380 nm.

Kinetics

βGal-1

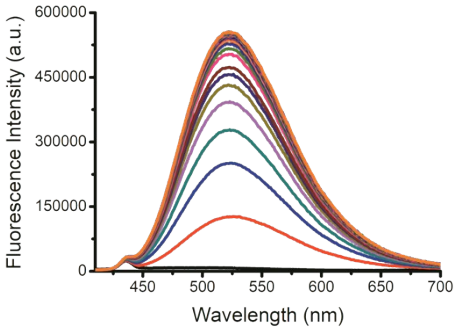
βGal-1 + βGal (0.05U/mL)



Kinetic constant pseudo first order = $0.0045 s^{-1}$ (Conversion = $1 - e^{-0.0045t}$)

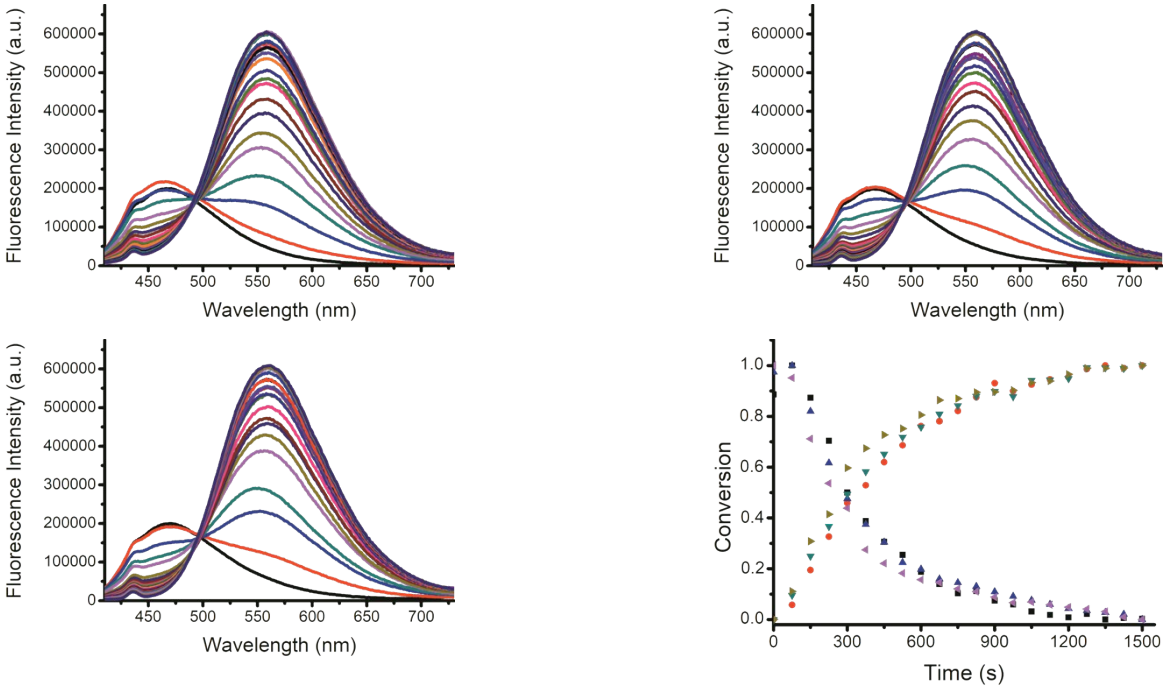
Equation	y = 1 - exp(-A*x)		
Adj. R-Square	0.9986		
		Value	Standard Error
mean bgal1	A	0.0045	4.52E-05

βGal-1 + βGal (0.05U/mL), HEPES buffer (20 mM, pH = 7.4)



βGal-3

βGal-3 + βGal (0.05U/mL)

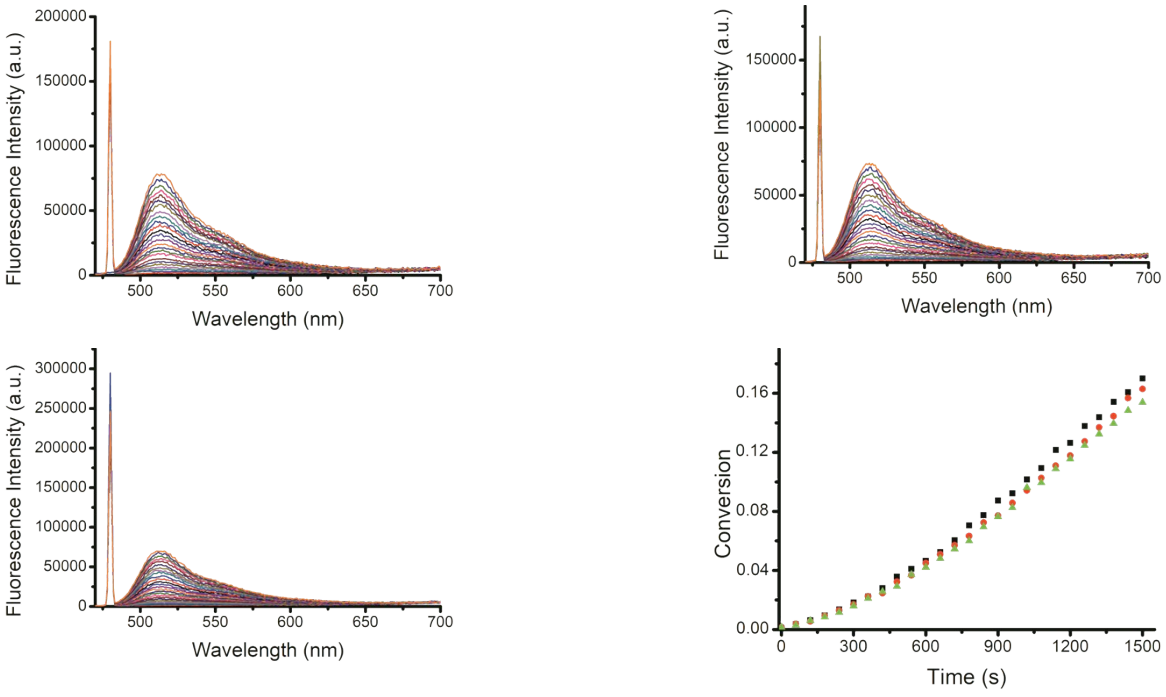


Kinetic constant pseudo first order = $0.0036 s^{-1}$ (Conversion = $1 - e^{-0.0036t}$)

Equation	y = 1 - exp(-A*x)		
Adj. R-Square	0.99845		
		Value	Standard Error
mean bgal-3	A	0.0036	4.04E-05

FDG

FDG + βGal (0.05U/mL)



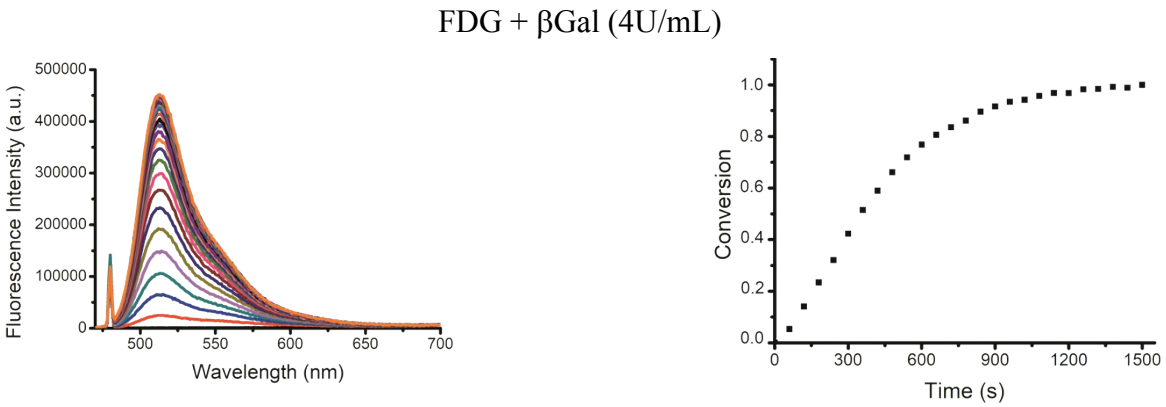


Fig S5. Fluorescence emission spectra for the kinetic experiments for **βGal-1**, **βGal-3** and FDG (black) Condition: dye concentration ≈ 0.2 μM; λ_{exc} = 380 nm, three independent experiments.

Chemical Stability and Selectivity

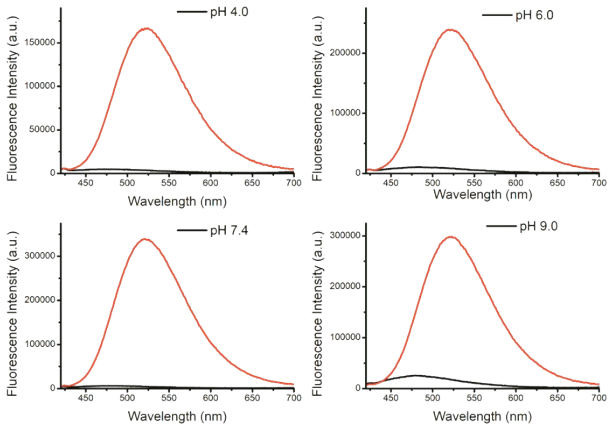


Fig S6. Fluorescence emission spectra of dye **2** (red) and **βGal-1** (black) in different pH after incubation during 96 hours. Condition: dye concentration ≈ 0.2 μM; λ_{exc} = 380 nm.

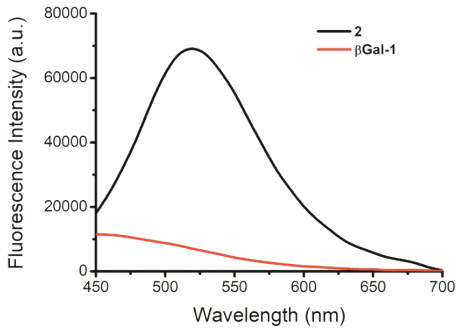


Fig S7. Fluorescence emission spectra of probe **βGal-1** (red) and the respective free dye **2** (black) in A-375 melanoma cells. Condition: probe concentration, 1 μM; λ_{exc} = 405 nm.

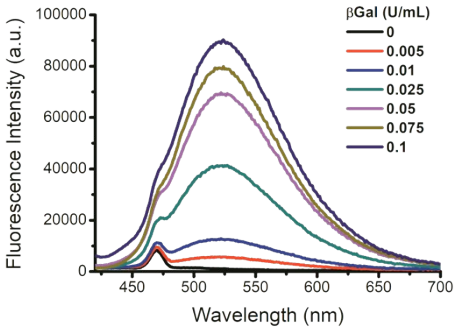


Fig S8. Fluorescence emission spectra of probe **βGal-1** in presence of cell lysate (1.46 μg/mL) with different amount of βGal. Condition: probe concentration, 0.2 μM; λ_{exc} = 405 nm.

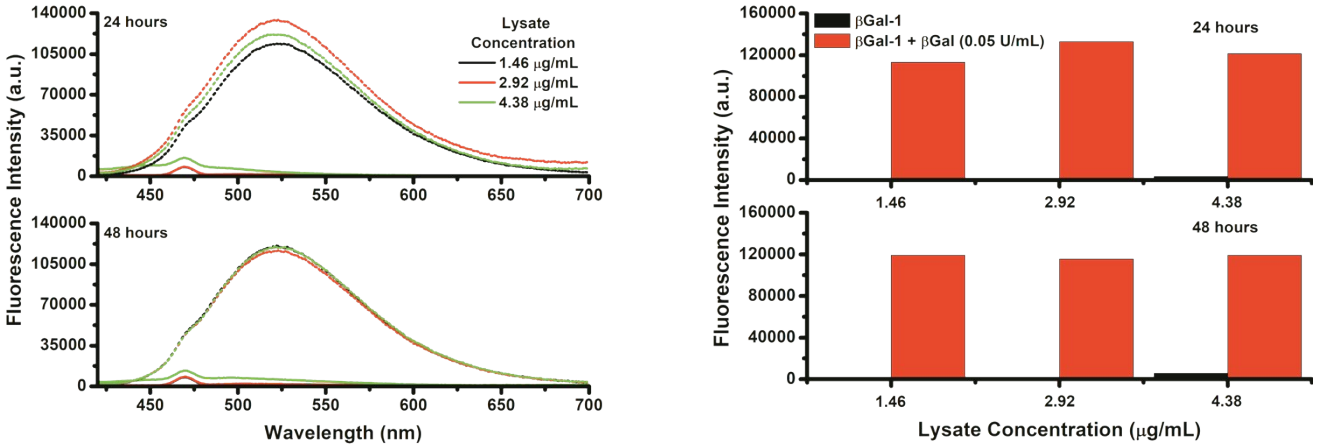


Fig S9. Fluorescence emission spectra of probe **βGal-1** in presence of different amount of cell lysate. Condition: probe concentration, 0.2 μM; λ_{exc} = 405 nm.

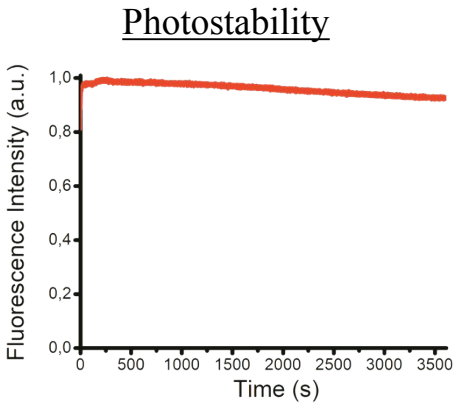


Fig S10. Fluorescence emission intensity of dye **2** under continuous irradiation in PBS solution during 1 hour. Condition: probe concentration, 0.2 μM; λ_{exc} = 405 nm.

Limit of Detection

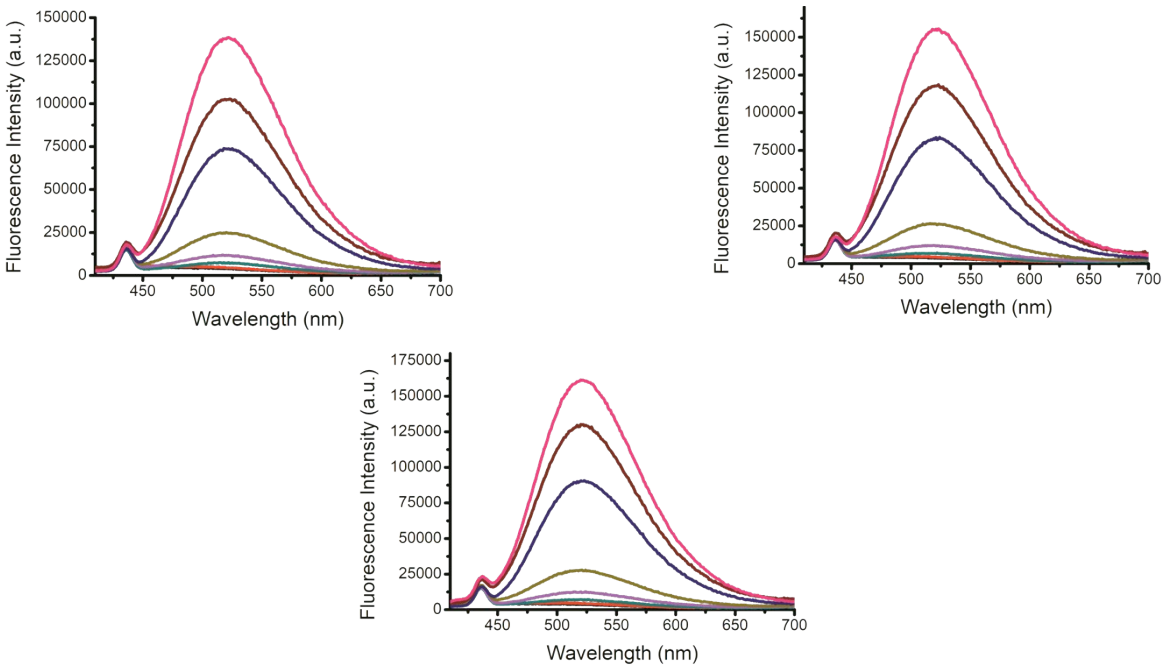


Fig S11. Fluorescence emission spectra of **βGal-1** in presence of different concentrations of β-Gal (0, 0.0005, 0.001, 0.00125, 0.0025, 0.005, 0.01, 0.025, 0.0375, 0.05 U/mL) for three independent experiments. Conditions: dye concentration ≈ 0.2 μM; λ_{exc}, 380 nm. Each spectrum was acquired after 10 minutes of reaction.

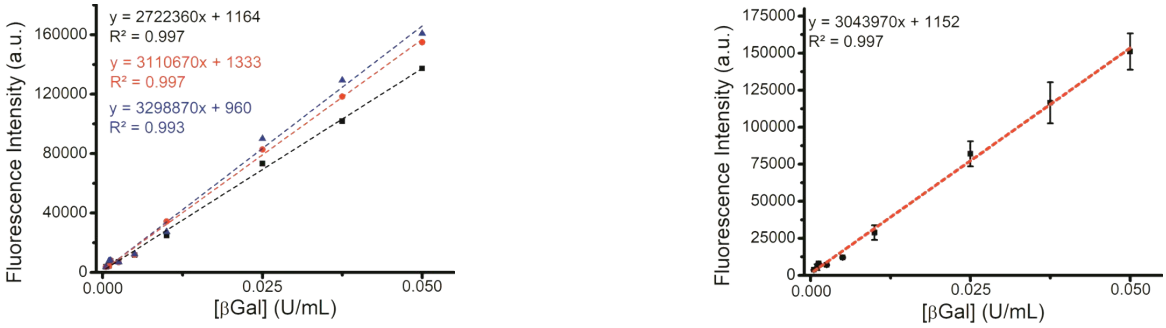
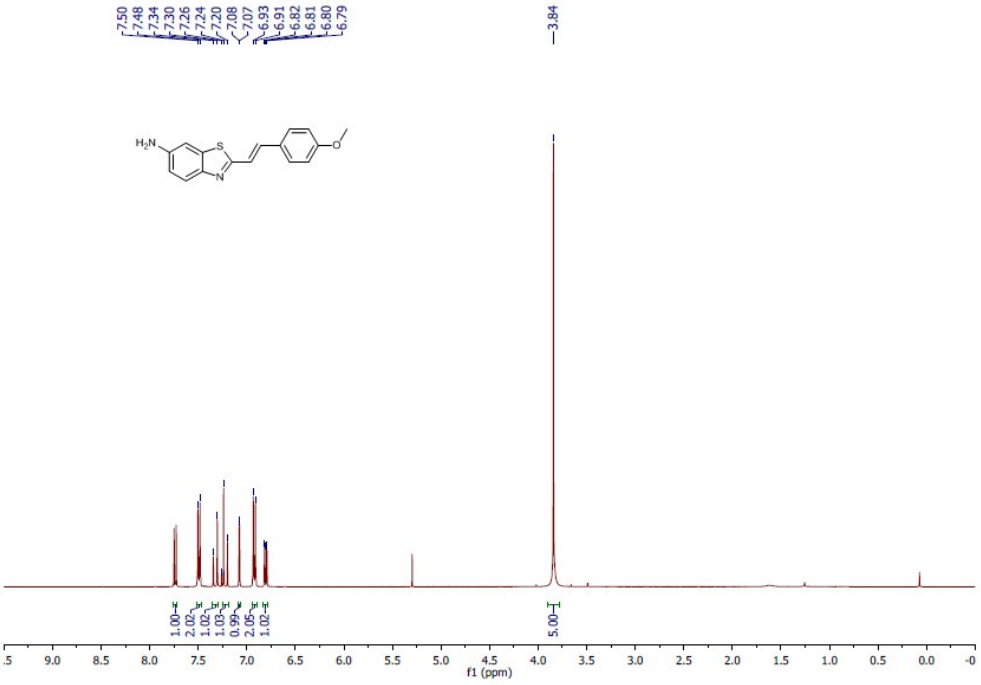
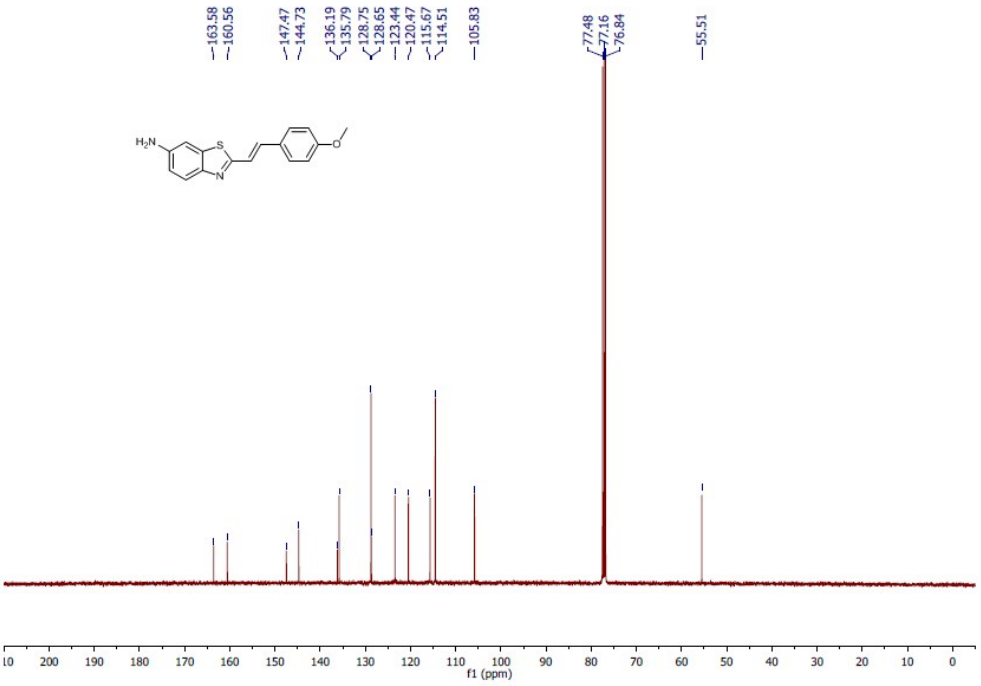


Fig S12. Fluorescence emission intensity of **βGal-1** in different concentrations of β-galactosidase in PBS (pH = 7.4) after 10 minutes for three independent experiments. Condition: dye concentration ≈ 0.2 μM; λ_{exc} = 380 nm.

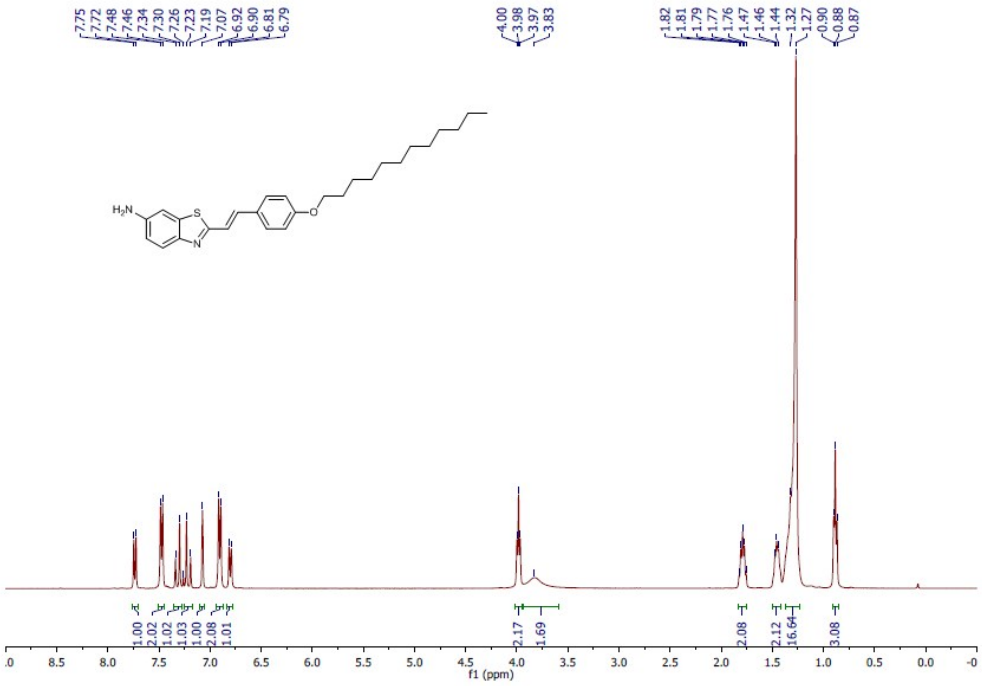
Spectroscopic Characterization



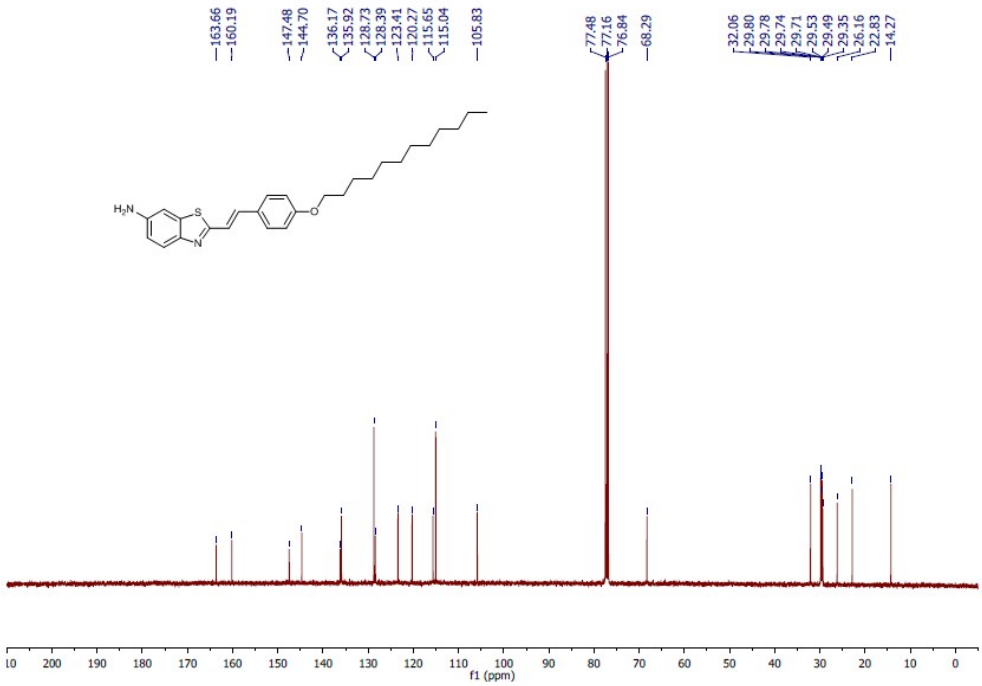
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2**.



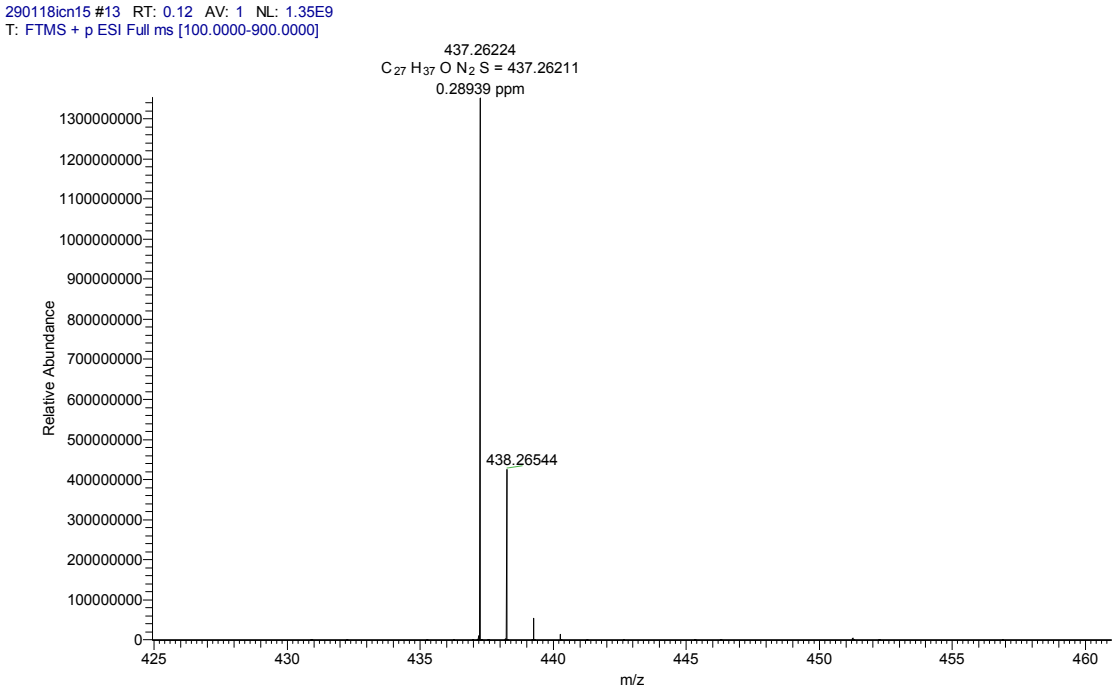
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2**.



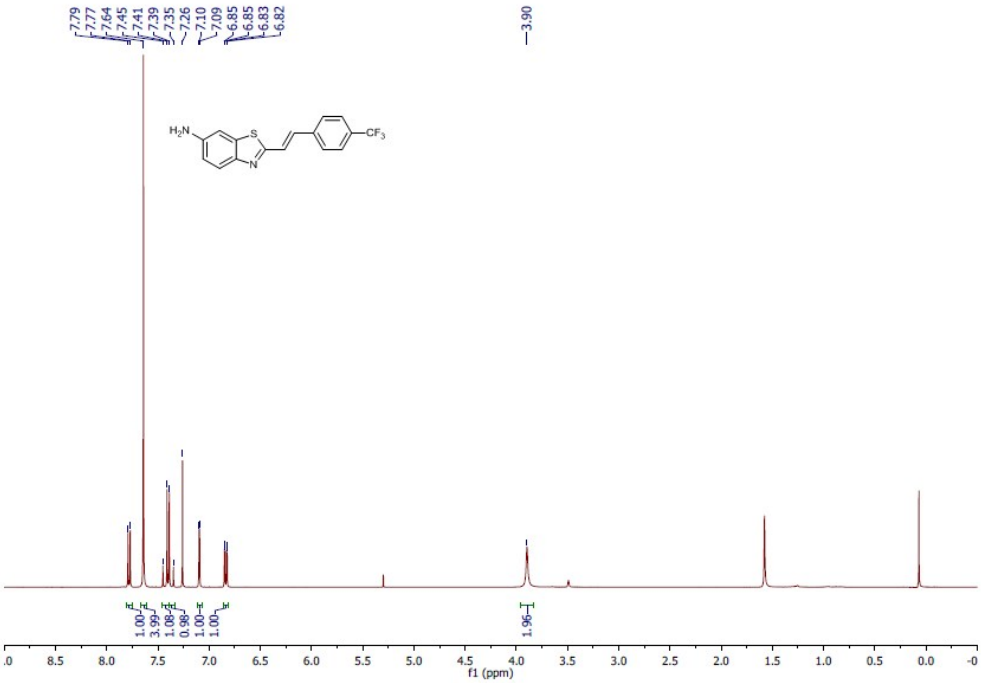
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3**.



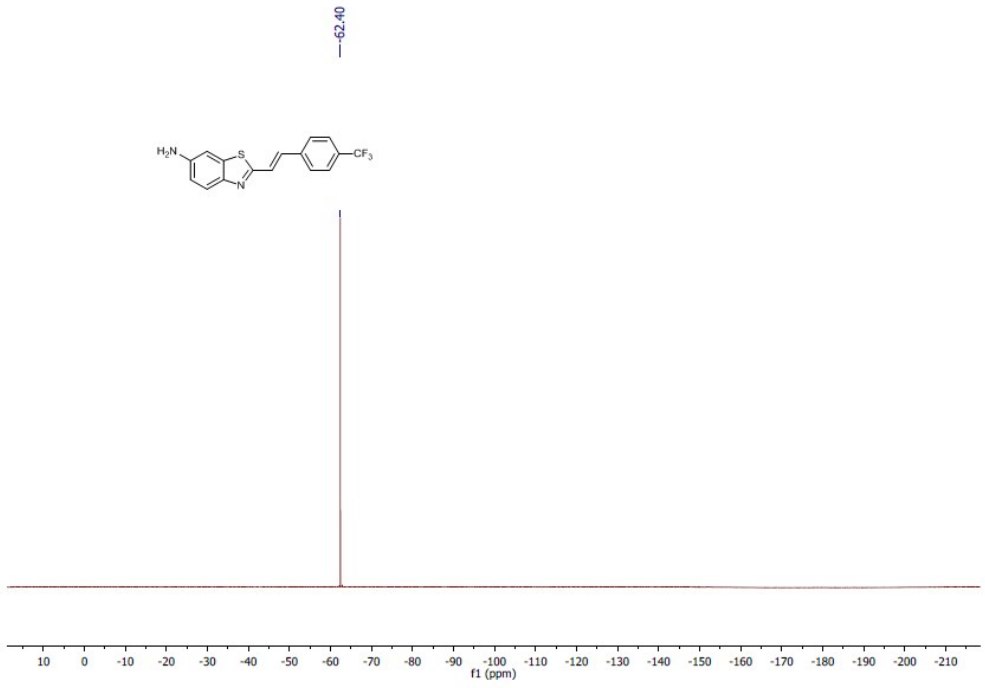
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **3**.



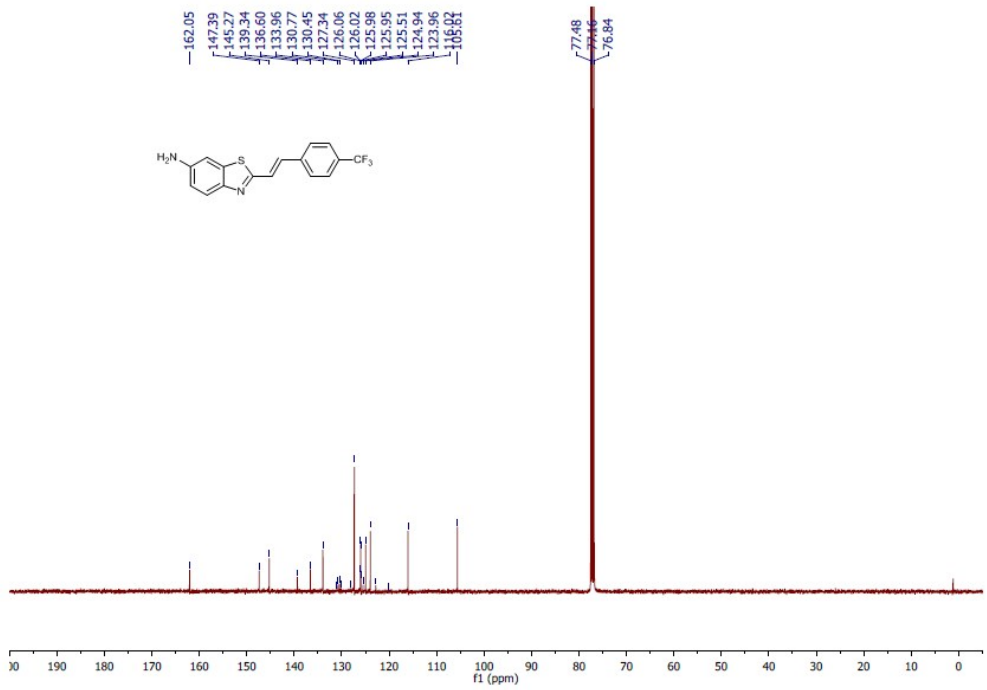
HRMS (ESI-MS) spectrum of **3**.



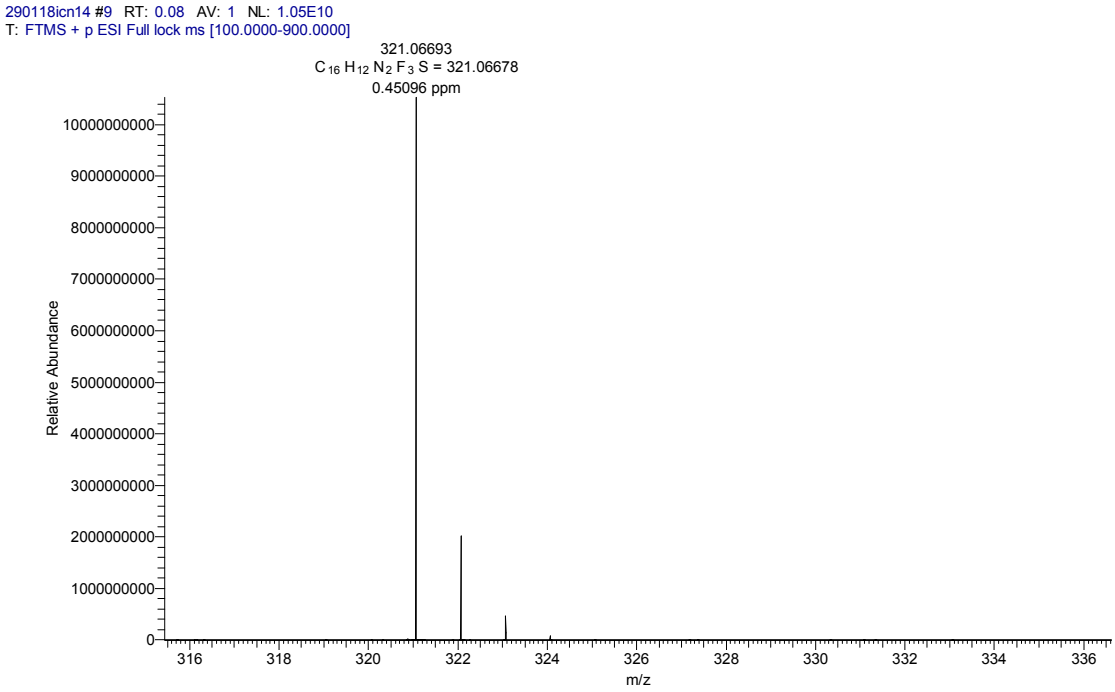
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **4**.



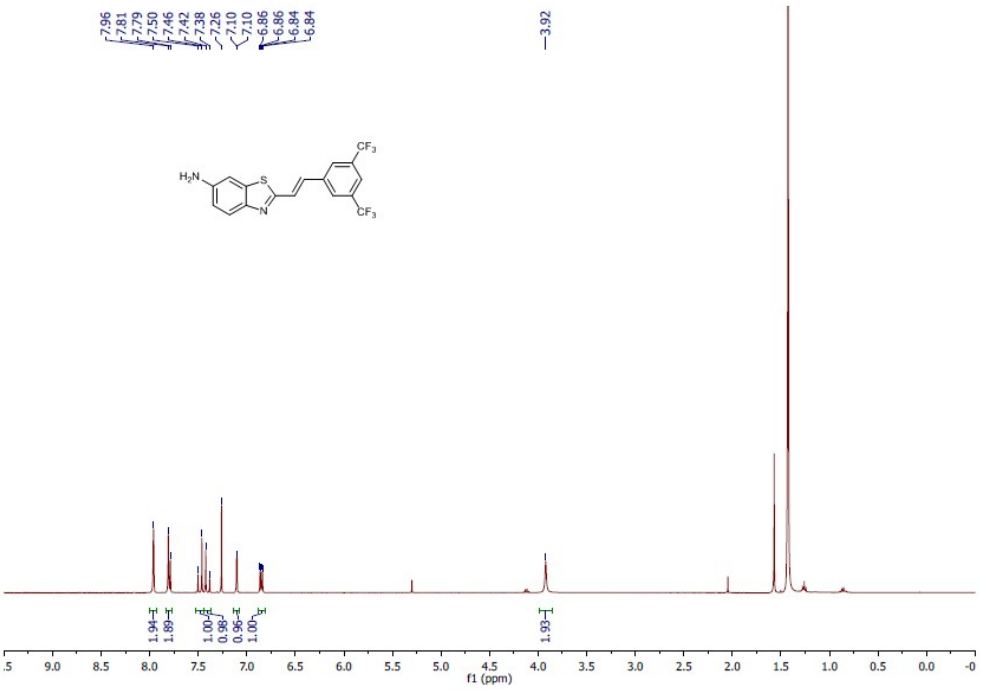
^{19}F NMR (376 MHz, $CDCl_3$) spectrum of **4.**



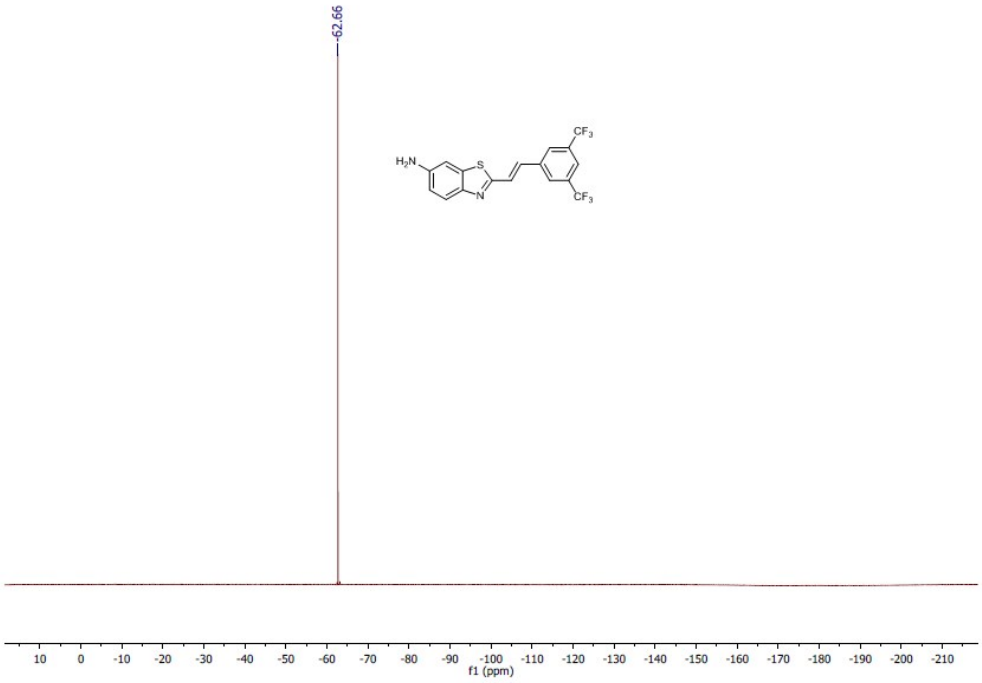
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **4**.



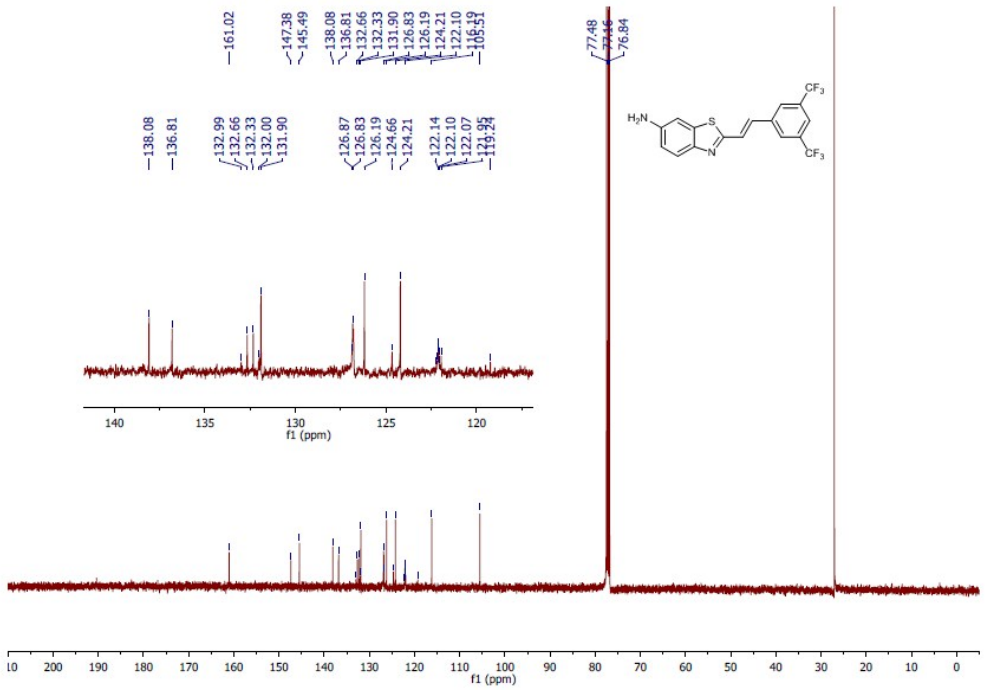
HRMS (ESI-MS) spectrum of **4**.



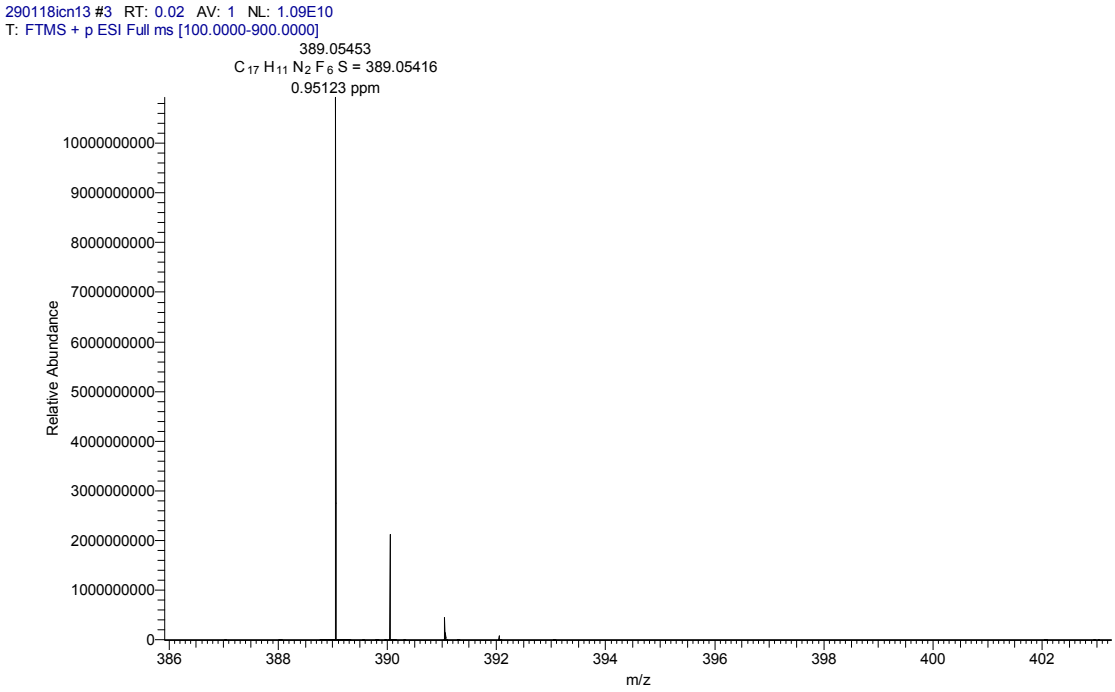
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **5**.



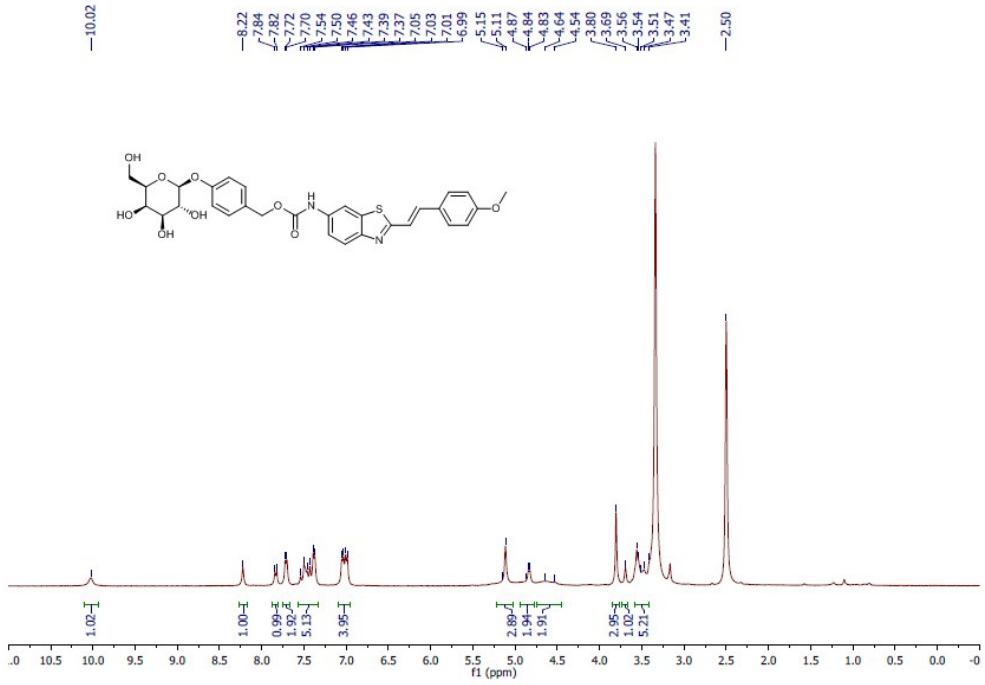
^{19}F NMR (376 MHz, $CDCl_3$) spectrum of **5.**



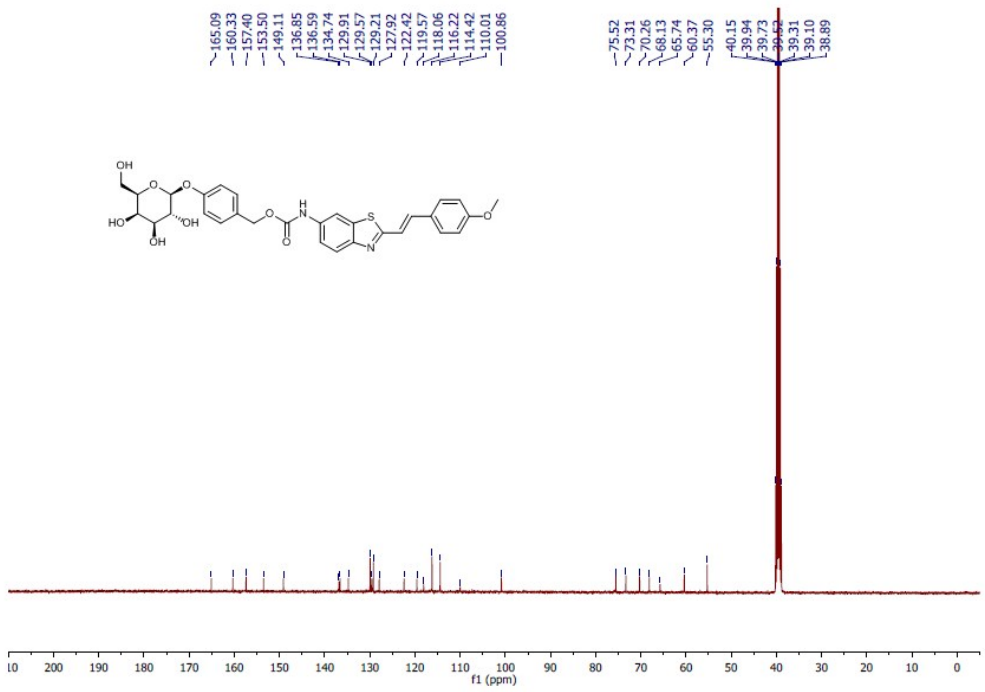
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **5**.



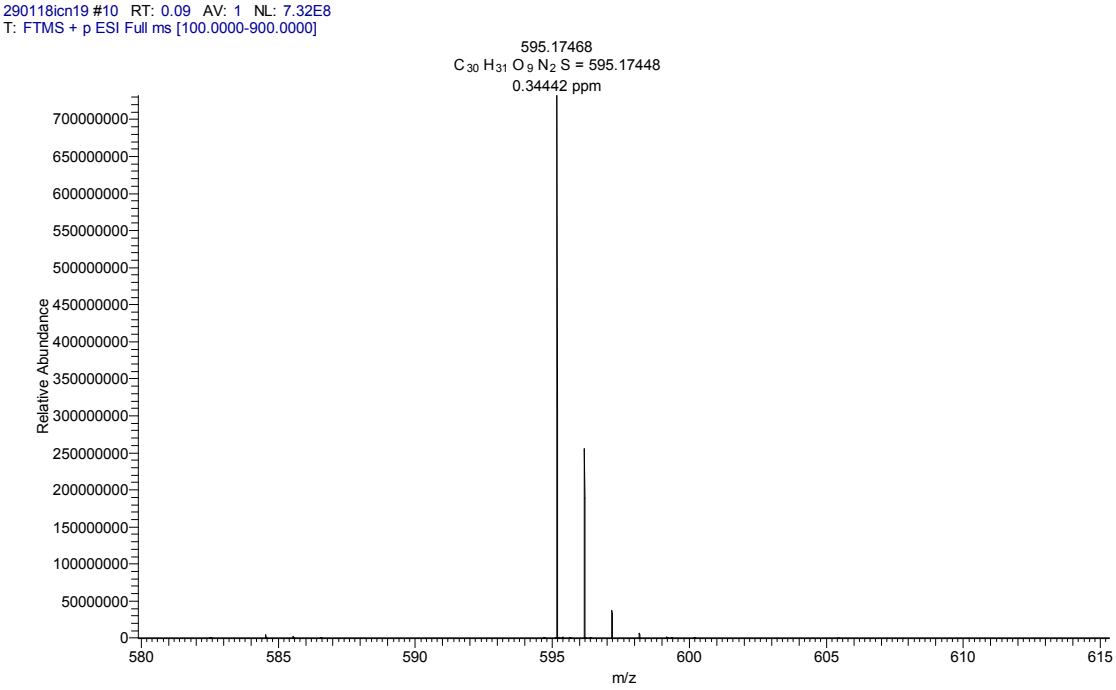
HRMS (ESI-MS) spectrum of **5**.



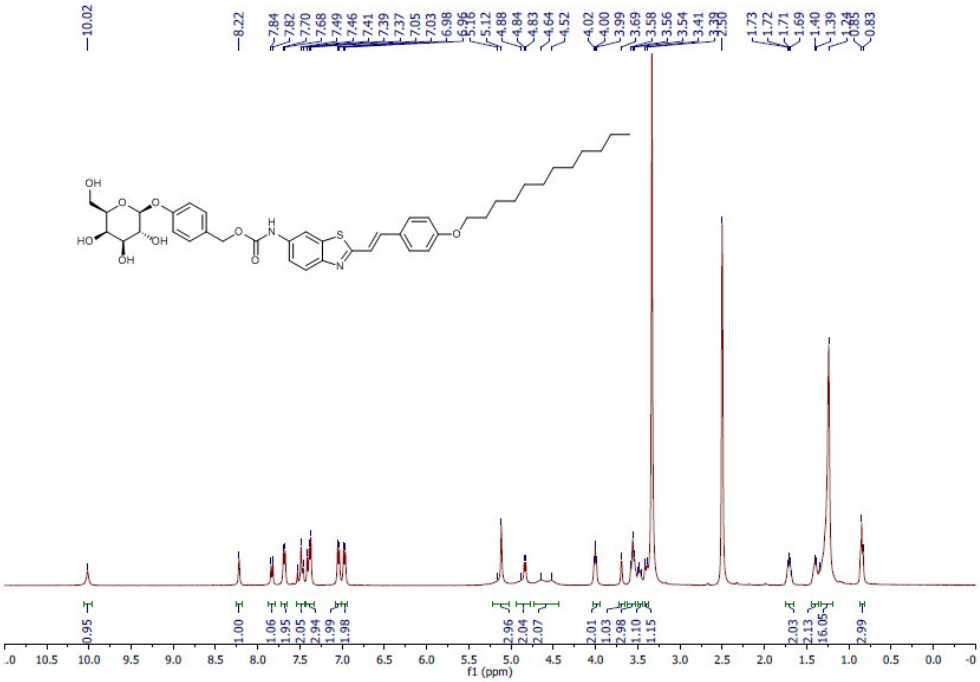
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **βGal-1**.



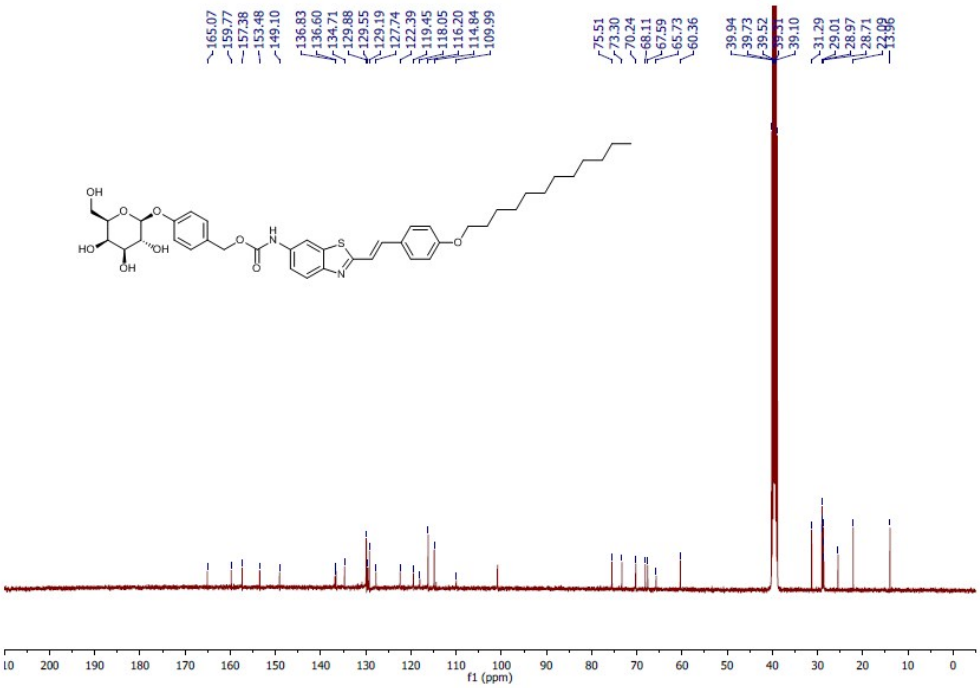
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of **βGal-1**.



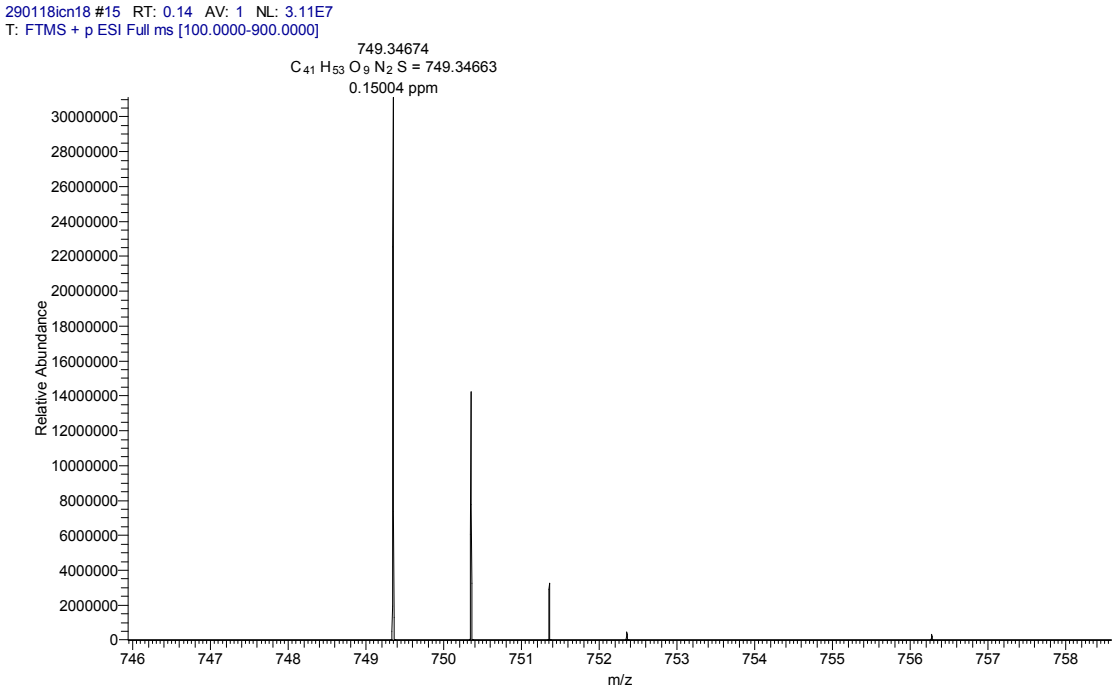
HRMS (ESI-MS) spectrum of **βGal-1**.



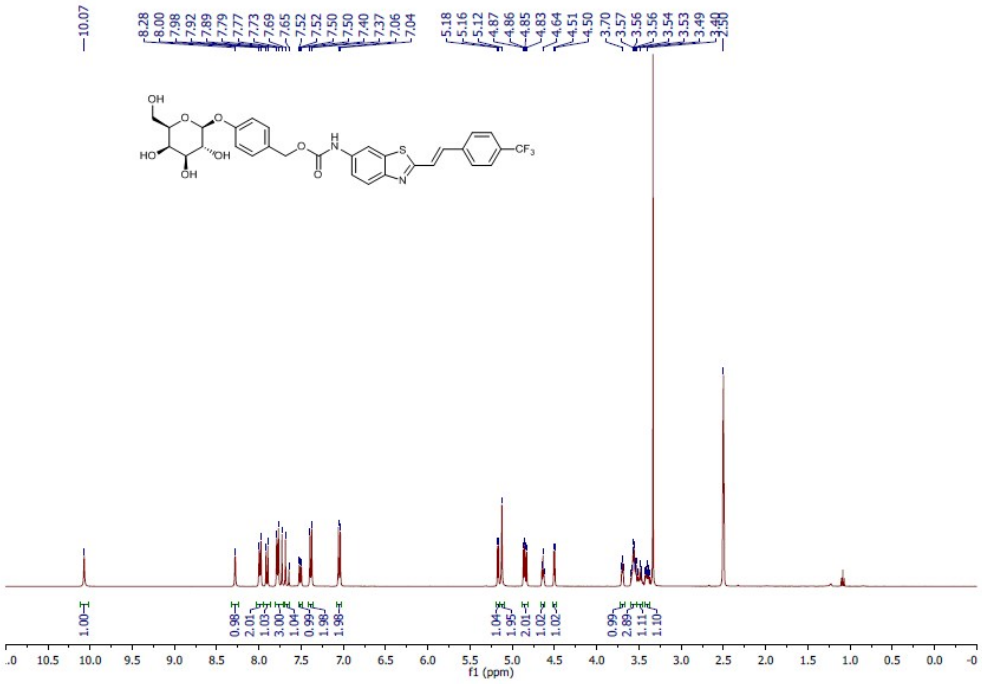
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **βGal-2**.



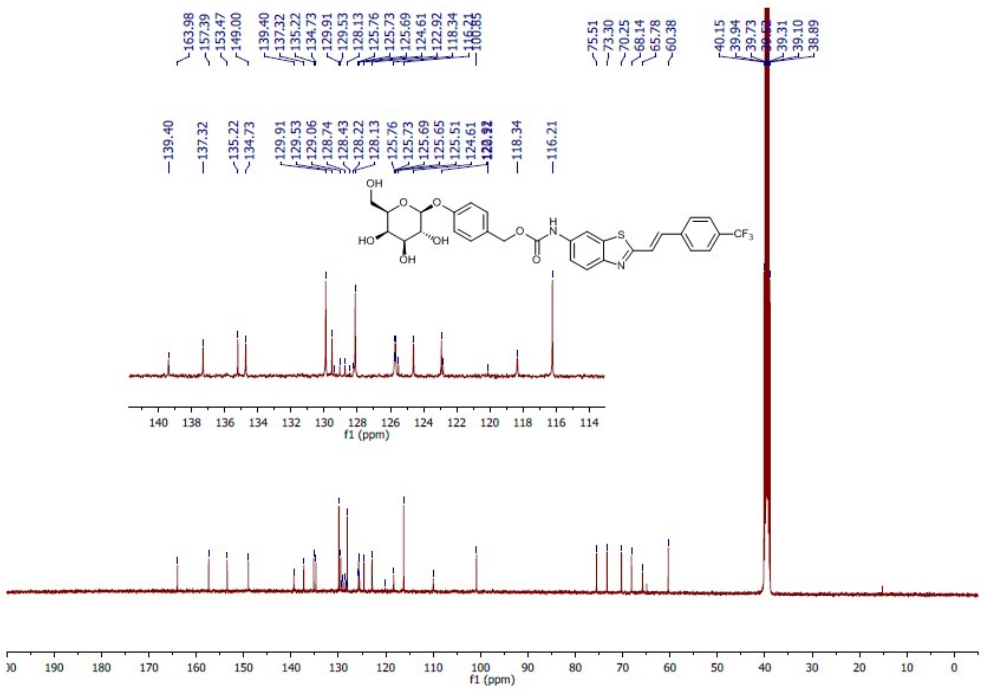
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of **βGal-2**.



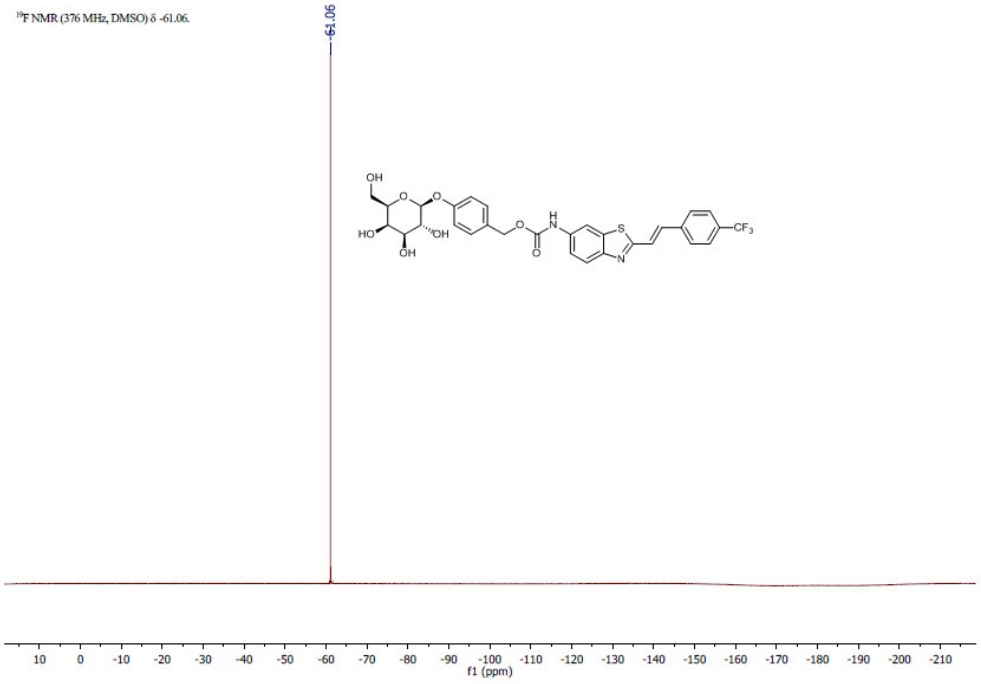
HRMS (ESI-MS) spectrum of **βGal-2**.



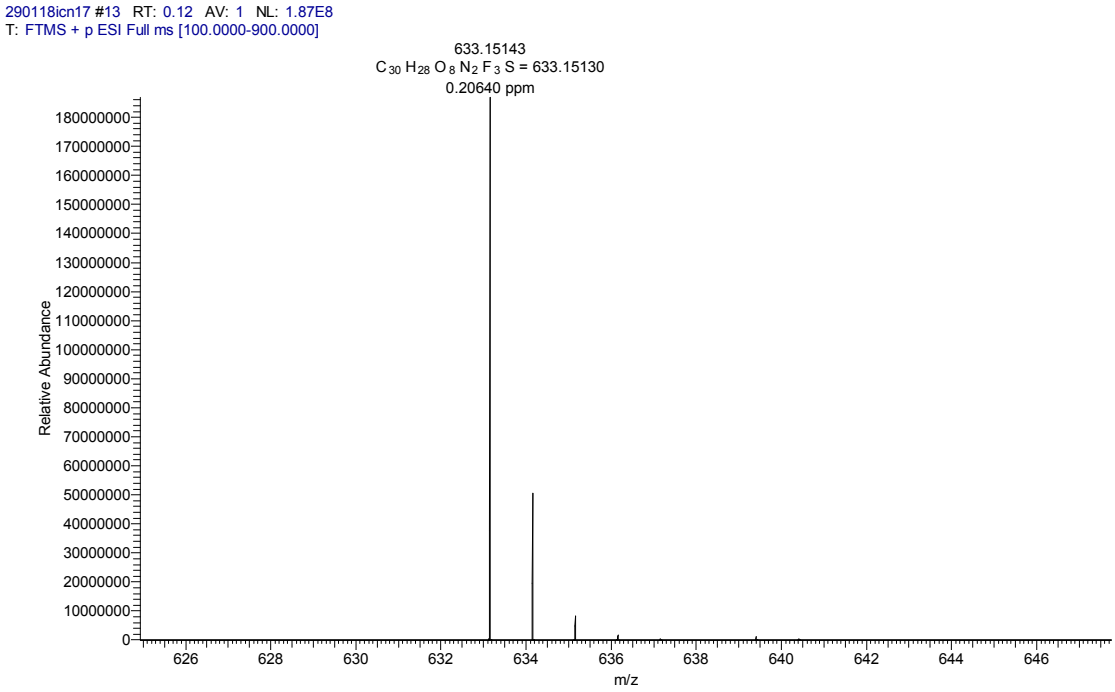
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **βGal-3**.



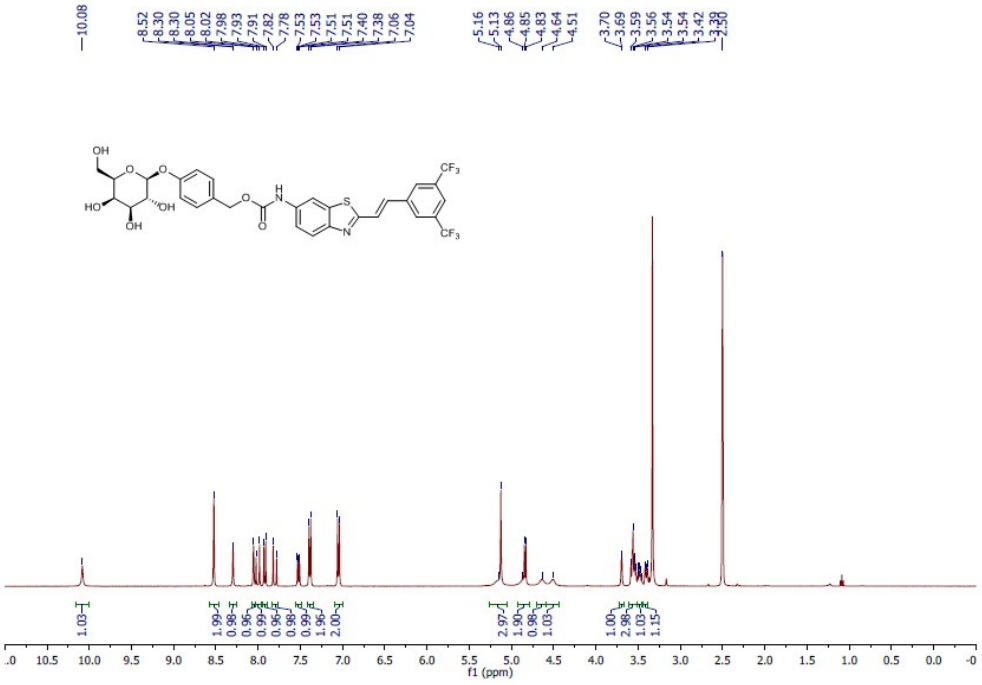
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of **βGal-3.**



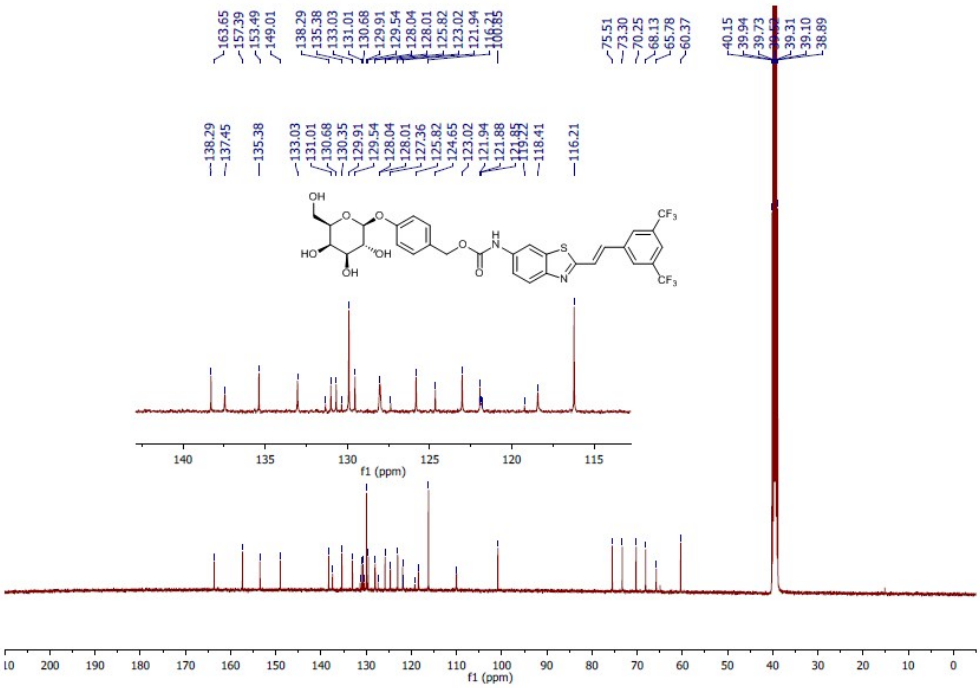
^{19}F NMR (376 MHz, DMSO-d_6) spectrum of **βGal-3.**



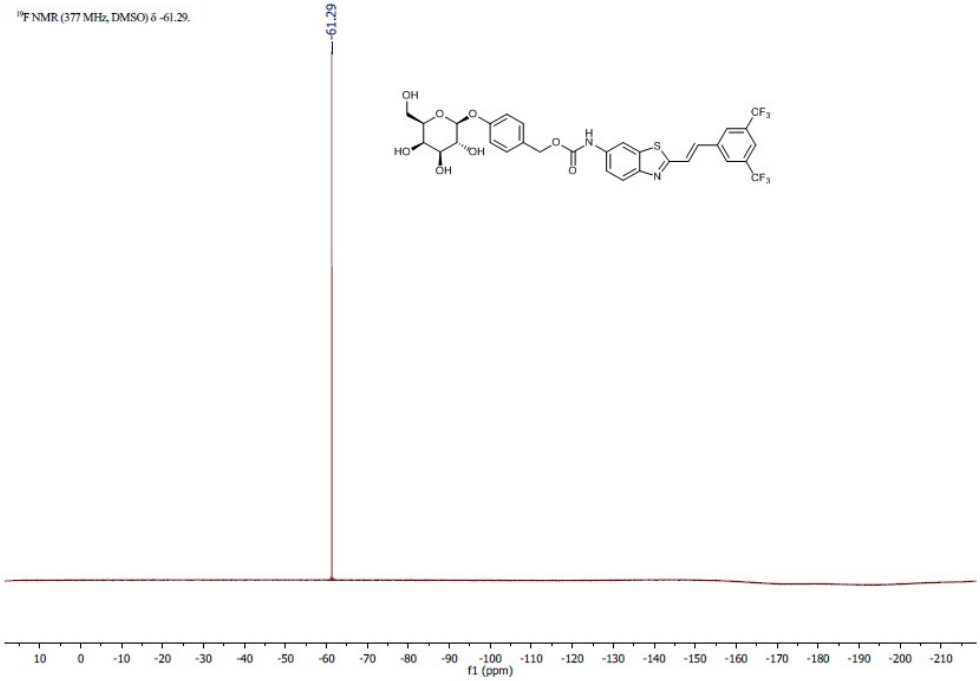
HRMS (ESI-MS) spectrum of **βGal-3**.



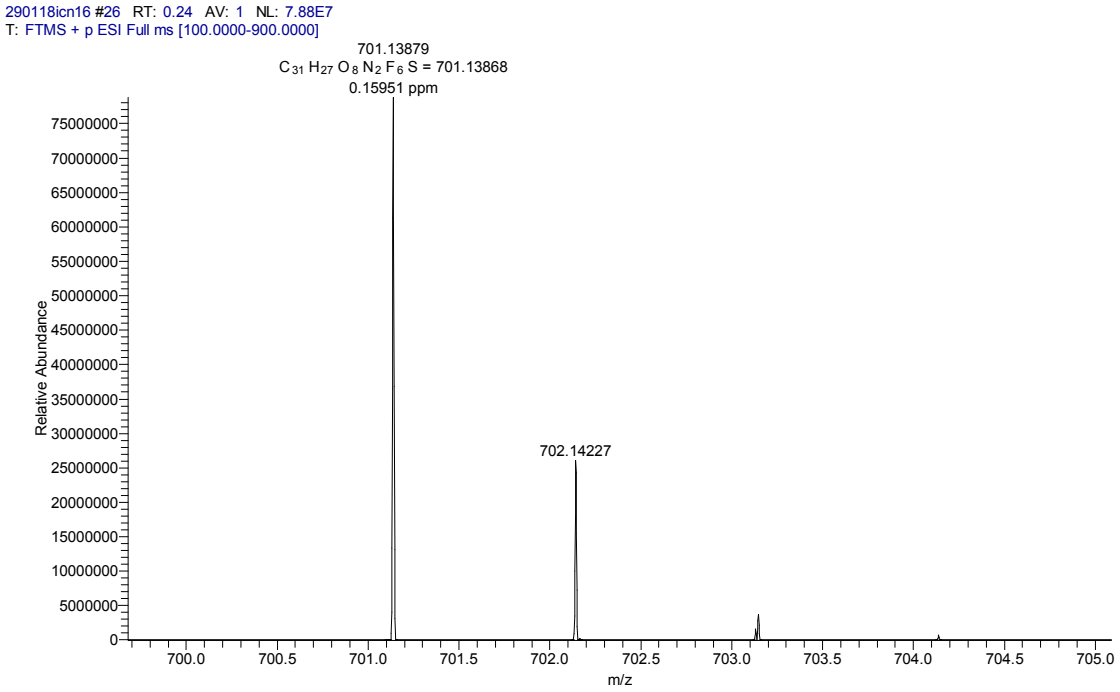
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **βGal-4**.



^{13}C NMR (100 MHz, DMSO-d_6) spectrum of **βGal-4**.



^{19}F NMR (376 MHz, DMSO-d_6) spectrum of **βGal-4**.



HRMS (ESI-MS) spectrum of **βGal-4**.