SUPPORTING INFORMATION

One-pot synthesis of fluorescein based β -aminoglycosylketones and their biological and material applications

Mani Rajasekar,^a and Thangamuthu Mohan Das*^{ab}

^aDepartment of Organic Chemistry, University of Madras, Guindy Campus, Chennai - 600 025, INDIA.

^bDepartment of Chemistry, School of Basic and Applied Sciences, Central University of Tamil Nadu (CUTN), Thiruvarur - 610 004, INDIA.

*Corresponding author: Department of Chemistry, School of Basic and Applied Sciences, Central University of Tamil Nadu (CUTN), Thiruvarur - 610 004, INDIA. E-mail address: <u>tmohandas@cutn.ac.in</u>;

Fax: 91-4366-225312; Tel: 91-9489054264

Experimental section

D-Glucose, butyraldehyde, resorcinol, potassium carbonate and other solvents were obtained from SRL, Chennai, Tamil Nadu, India. Column chromatography was performed on Silica Gel (100-200 mesh). Melting points were measured on Sigma micro melting point apparatus and are uncorrected. NMR spectra were recorded on Bruker DRX 300 MHz in CDCl₃ or DMSO- d_6 at University of Madras (Chennai,Tamil Nadu, India). TMS was used as the internal standard ($\delta = 0.00$ ppm) and all the *J* values are given in hertz. Optical rotation was performed by using Rudolph-Autopol II digital polarimeter. Elemental analyses were performed using Perkin-Elmer 2400 elemental analyzer. FESEM images were recorded on a Hitachi SU-6600 instrument and HRTEM was recorded using a JEOL, JEM 3010 model (LaB6 filament). Thermal transitions for gelators and gels were determined on a NETZSCH DSC 204 instrument. Rheological studies were recorded in Gemini 2000 using pp40. X-ray diffractograms of the dried films were recorded on XRD RINT 2500 diffractometer using Ni filtered Cu K α radiation.

In vitro antioxidant activity

In vitro antioxidant activity for fluorescein based β -aminoglycosylketones **15-20** were evaluated by the DPPH radical scavenging method.³² The principle of this assay is based on the measurement of the scavenging ability of the antioxidant towards the stable radical. The free radical of DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors, and its stability is evaluated by the decolouration assay, which evaluates the decrease in absorbance at 517 nm produced by the addition of the antioxidant to a DPPH solution in ethanol. Assays were performed in 1.5 mL reaction mixtures containing 1 mL of 0.1 mM DPPH ethanol solution and 1 mL of different concentrations of fluorescein based β -aminoglycosylketones **15-20** (20-200 µg/mL)/0.5 mL of ethanol (as control). After 30 min of incubation at 37 °C in the dark, the absorbance of the reaction mixtures were measured at 517 nm. IC₅₀ is the concentration of the sample required to scavenge 50% of the DPPH free radicals. The percentage of inhibition is calculated by subtracting the absorbance of the sample from the absorbance

of the control divided by absorbance of the control. The lower the IC_{50} , the higher is the antioxidant activity of the examined compound. From the difference in the absorbance of DPPH, the percentage of inhibition was calculated as a function of antioxidant activity.

General procedure for the synthesis of fluorescein based β -aminoglycosylketone derivatives (15-20)

To a solution of the fluorescein-monoaldehyde 7, (1.0 mmol), aromatic amines 8-13 (1.0 mmol), 1-(4,6-O-butylidene- β -D-glucopyranosyl)propan-2-one 14, (1.0 mmol) and dry MeOH (10 mL) were added potassium carbonate (0.1 mmol). After stirring at 50 °C for a given period of time, the reaction mixture was evaporated under reduced pressure and extracted by chloroform-water. The chloroform layer was dried over anhyd.Na₂SO₄ and concentrated to dryness. The product was further purified by flash column chromatography.

Physicochemical and spectral data for 3-(fluorescein)-2-(4,6-*O*-butylidene-β-Dglucopyranosyl)-3-(phenylamino)-butan-1-one (15)



Compound, **15** was obtained by the reaction of fluorescein-monoaldehyde **7**, (1.0 mmol, 0.36 g), aniline **8**, (1.0 mmol, 0.2 mL), 1-(4,6-*O*-butylidene- β -D-glucopyranosyl)-propan-2-one **14**, (1.0 mmol, 0.12 g) as pale yellow solid: Yield: 0.50 g (71%); mp 156-158 °C; $[\alpha]_D^{31}$ + 87.8 (c 0.1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 0.92 (t, 3H, J = 7.3 Hz, -CH₃), 1.42 (q, 2H, J = 9.2 Hz, -CH₂), 1.62 (q, 2H, J = 9.0 Hz, -CH₂), 2.20 (s, 3H, -COCH₃), 2.67 (dd, 3H, J = 7.8 Hz, J = 7.8 Hz, Sac-H), 2.91 (d, 1H, J = 16.5 Hz, -CH), 3.22 (t, 1H, J = 9.0 Hz, Sac-H), 3.51 (d, 1H, J = 7.1 Hz, -CH), 3.69 (t, 1H, J = 8.4 Hz, Sac-H),

3.83 (s, 2H, Sac-OH), 4.13 (d, 1H, J = 7.2 Hz, Ano-H), 4.53 (t, 1H, J = 4.8 Hz, Sac-H), 6.59 (s, 1H, -NH), 6.77 (d, 2H, J = 8.4 Hz, Ar-H), 7.06 (d, 2H, J = 8.4 Hz, Ar-H), 7.14 (d, 2H, J = 7.2 Hz, Ar-H), 7.26 (t, 3H, J = 8.0 Hz, Ar-H), 7.34 (s, 2H, Ar-OH), 7.64-7.73 (m, 4H, Ar-H), 8.06 (d, 1H, J = 7.2 Hz, Ar-H). ¹³C NMR (75 MHz, CDCl₃): δ 207.4, 169.0, 152.7, 151.1, 136.4, 135.5, 132.0, 130.2, 129.0, 125.8, 125.4, 124.7, 123.7, 117.4, 117.0, 110.6, 102.4, 81.2, 80.4, 76.0, 75.1, 74.2, 70.5, 68.2, 59.6, 36.2, 30.9, 28.0, 17.4, 13.9. ESI-MS: calc. for C40H39NO11, 709.74; m/z found, 710.64 [M + H]⁺; elemental Anal. Found: C, 67.84; H, 5.74; N, 1.87. Calc. For C₄₀H₃₉NO₁₁: C, 67.82; H, 5.70; N, 1.81.

Physicochemical and spectral data for 3-(fluorescein)-2-(4,6-*O*-butylidene-β-Dglucopyranosyl)-3-(4-bromophenylamino)-butan-1-one (16)



Compound, **16** was obtained by the reaction of fluorescein-monoaldehyde **7**, (1.0 mmol, 0.36 g), 4-bromoaniline **9**, (1.0 mmol, 0.17 g), 1-(4,6-*O*-butylidene- β -D-glucopyranosyl)-propan-2-one **14**, (1.0 mmol, 0.12 g) as brown solid: Yield: 0.48 g (62%); mp 162-164 °C; $[\alpha]_D^{31}$ + 58.5 (c 0.1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 0.92 (t, 3H, J = 7.4 Hz, -CH₃), 1.42 (q, 2H, J = 11.0 Hz, -CH₂), 1.62 (q, 2H, J = 9.1 Hz, -CH₂), 2.19 (s, 3H, -COCH₃), 2.54-2.67 (m, 1H, Sac-H), 2.90 (d, 1H, J = 11.2 Hz, -CH), 3.19-3.45 (m, 4H, Sac-H), 3.52 (d, 1H, J = 6.7 Hz, -CH), 3.67 (t, 1H, J = 8.7 Hz, Sac-H), 3.80 (d, 1H, J = 7.5 Hz, Ano-H), 4.12 (d, 1H, J = 3.6 Hz, Sac-H), 4.52 (t, 2H, J = 4.8 Hz, Sac-OH), 6.71 (s, 1H, -NH), 6.77 (t, 3H, J = 6.8 Hz, Ar-H), 7.05 (dd, 2H, J = 8.4 Hz, J = 8.4 Hz, Ar-H), 7.14 (t, 4H, J = 7.5 Hz, Ar-H), 7.34 (s, 2H, Ar-OH), 7.63-7.73 (m, 3H, Ar-H), 8.06 (d, 1H, J = 7.2 Hz, Ar-H). ¹³C NMR (75 MHz, CDCl₃): δ 207.3, 169.1, 152.8, 151.2, 145.7, 136.4, 135.5,

130.3, 129.0, 125.8, 125.4, 124.7, 123.7, 119.0, 117.4, 115.5, 102.5, 81.2, 80.5, 77.5, 77.0, 76.6, 76.0, 75.1, 74.2, 70.5, 68.2, 59.7, 36.2, 30.9, 29.5, 17.4, 13.9. ESI-MS: calc. for C40H38BrNO11, 788.63; m/z found, 789.42 [M + H]⁺; elemental anal. Found: C, 60.72; H, 4.76; N, 1.88. Calc. for $C_{40}H_{38}BrNO_{11}$: C, 60.79; H, 4.71; N, 1.82.

Physicochemical and spectral data for 3-(fluorescein)-2-(4,6-*O*-butylidene-β-Dglucopyranosyl)-3-(4-nitrophenylamino)-butan-1-one (17)



Compound, **17** was obtained by the reaction of fluorescein-monoaldehyde **7**, (1.0 mmol, 0.36 g), 4-nitroaniline **10**, (1.0 mmol, 0.13 g), 1-(4,6-O-butylidene- β -D-glucopyranosyl)-propan-2-one **14**, (1.0 mmol, 0.12 g) as pale yellow solid: Yield: 0.46 g (61%); mp 170-172 °C; [α]_D³¹ + 77.2 (c 0.1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 0.91 (t, 3H, J = 7.2 Hz, -CH₃), 1.42 (q, 2H, J = 9.8 Hz, -CH₂), 1.60 (q, 2H, J = 9.0 Hz, -CH₂), 2.19 (s, 3H, -COCH₃), 2.65 (dd, 1H, J = 6.7 Hz, J = 6.6 Hz, Sac-H), 2.88 (d, 1H, J = 11.5 Hz, -CH), 3.19-3.45 (m, 5H, Sac-H), 3.52 (d, 1H, J = 6.9 Hz, -CH), 4.12 (q, 2H, J = 8.4 Hz, Sac-H), 4.52 (d, 2H, J = 8.7 Hz, Ano-H), 6.62 (s, 1H, -NH), 6.77 (d, 2H, J = 8.4 Hz, Ar-H), 7.05 (d, 2H, J = 8.4 Hz, Ar-H), 7.15 (d, 4H, J = 6.9 Hz, Ar-H), 7.34 (s, 2H, Ar-OH), 7.64-7.73 (m, 2H, Ar-H), 8.06 (d, 3H, J = 8.7 Hz, Ar-H). ¹³C NMR (75 MHz, CDCl₃): δ 207.3, 169.2, 152.9, 152.6, 151.2, 138.7, 136.4, 135.6, 130.3, 129.0, 126.3, 125.8, 125.4, 124.7, 123.7, 117.4, 117.3, 113.3, 102.4, 81.3, 80.4, 77.5, 75.2, 74.3, 70.5, 68.2, 59.6, 36.2, 30.9, 29.8, 17.5, 13.9. Elemental Anal. Found: C, 63.56; H, 5.37; N, 3.61. Calc. for C₄₀H₃₈N₂O₁₃: C, 63.51; H, 5.32; N, 3.66.

Physicochemical and spectral data for 3-(fluorescein)-2-(4,6-*O*-butylidene-β-Dglucopyranosyl)-3-(4-chlorophenylamino)-butan-1-one (18)



Compound, 18 was obtained by the reaction of fluorescein-monoaldehyde 7, (1.0 mmol, 0.36 g), 4-chloroaniline 11, (1.0 mmol, 0.12 g), $1-(4,6-O-butylidene-\beta-D$ glucopyranosyl)-propan-2-one 14, (1.0 mmol, 0.12 g) as pale yellow solid: Yield: 0.40 g (54%); mp 148-150 °C; $[\alpha]_D^{31}$ + 56.8 (c 0.1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 0.92 $(t, 3H, J = 7.2 Hz, -CH_3), 1.43 (q, 2H, J = 9.0 Hz, -CH_2), 1.63 (q, 2H, J = 5.2 Hz, -CH_2),$ 2.20 (s, 3H, -COCH₃), 2.65 (dd, 1H, J = 8.1 Hz, J = 8.1 Hz, Sac-H), 2.90 (d, 1H, J = 11.2Hz, -CH), 3.22 (t, 1H, J = 9.0 Hz, Sac-H), 3.28-3.45 (m, 4H, Sac-H), 3.57 (d, 1H, J = 6.9Hz, -CH), 3.69 (t, 1H, J = 8.7 Hz, Sac-H), 3.82 (d, 1H, J = 2.7 Hz, Sac-H), 4.12 (d, 1H, J= 8.7 Hz, Ano-H), 4.52 (t, 1H, J = 7.8 Hz, Sac-OH), 6.48 (t, 2H, J = 7.8 Hz, Ar-H), 6.77 (q, 4H, J = 7.4 Hz, Ar-H), 7.05 (s, 1H, -NH), 7.15 (q, 2H, J = 7.2 Hz, Ar-H), 7.34 (s, 2H, J)Ar-OH), 7.67 (q, 4H, J = 9.6 Hz, Ar-H), 8.06 (d, 1H, J = 7.2 Hz, Ar-H). ¹³C NMR (75) MHz, CDCl₃): δ 207.1, 169.0, 152.8, 151.2, 146.7, 138.9, 135.5, 130.3, 129.0, 125.4, 124.7, 123.7, 119.9, 117.4, 114.7, 102.5, 84.2, 81.2, 80.4, 77.5, 77.0, 76.6, 75.9, 75.2, 74.3, 70.6, 68.2, 60.7, 36.2, 30.9, 28.9, 17.5, 13.9. ESI-MS: calc. for C40H38CINO11, 743.21; m/z found, 744.39 [M + H]⁺; elemental Anal. Found: C, 64.76; H, 5.25; N, 1.68. Calc. for C₄₀H₃₈ClNO₁₁: C, 64.71; H, 5.29; N, 1.63.

Physicochemical and spectral data for 3-(fluorescein)-2-(4,6-*O*-butylidene-β-Dglucopyranosyl)-3-(4-fluorophenylamino)-butan-1-one (19)



Compound, **19** was obtained by the reaction of fluorescein-monoaldehyde **7**, (1.0 mmol, 0.36 g), 4-fluoroaniline **12**, (1.0 mmol, 0.2 mL), 1-(4,6-O-butylidene- β -D-glucopyranosyl)-propan-2-one **14**, (1.0 mmol, 0.12 g) as pale yellow solid: Yield: 0.38 g (52%); mp 166-168 °C; $[\alpha]_D^{31}$ + 34.7 (c 0.1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 0.92 (t, 3H, J = 7.2 Hz, -CH₃), 1.42 (q, 2H, J = 9.2 Hz, -CH₂), 1.62 (q, 2H, J = 8.5 Hz, -CH₂), 2.19 (s, 3H, -COCH₃), 2.64 (q, 1H, J = 8.1 Hz, Sac-H), 2.91 (d, 1H, J = 10.2 Hz, -CH), 3.19-3.45 (m, 4H, Sac-H), 3.52 (d, 1H, J = 6.6 Hz, -CH), 3.69 (t, 1H, J = 8.7 Hz, Sac-H), 3.81 (d, 1H, J = 8.1 Hz, Ano-H), 4.12 (d, 1H, J = 5.7 Hz, Sac-H), 4.53 (t, 2H, J = 4.5 Hz, Sac-OH), 6.76 (s, 1H, -NH), 7.04 (d, 3H, J = 7.5 Hz, Ar-H), 7.14 (d, 4H, J = 7.2 Hz, Ar-H), 7.34 (s, 2H, Ar-OH), 7.64-7.73 (m, 5H, Ar-H), 8.05 (d, 1H, J = 7.2 Hz, Ar-H). ¹³C NMR (75 MHz, CDCl₃): δ 207.3, 169.1, 152.8, 151.2, 136.4, 135.5, 130.3, 130.2, 129.0, 126.9, 125.8, 125.5, 124.7, 123.6, 119.4, 117.4, 115.6, 102.5, 81.2, 80.4, 75.2, 74.3, 70.5, 68.2, 58.5, 36.2, 32.0, 30.9, 17.4, 17.3, 13.9. Elemental Anal. Found: C, 66.27; H, 5.46; N, 1.72. Calc. for C₄₀H₃₈FNO₁₁: C, 66.22; H, 5.42; N, 1.77.

Physicochemical and spectral data for 3-(fluorescein)-2-(4,6-*O*-butylidene-β-Dglucopyranosyl)-3-(4-methoxy phenylamino)-butan-1-one (20)



Compound, **20** was obtained by the reaction of fluorescein-monoaldehyde **7**, (1.0 mmol, 0.36 g), 4-methoxyaniline **13**, (1.0 mmol, 0.12 g), 1-(4,6-*O*-butylidene- β -D-glucopyranosyl)-propan-2-one **14**, (1.0 mmol, 0.12 g) as brown solid: Yield: 0.50 g (68%); mp 160-162 °C; [α]_D³¹ + 23.6 (c 0.1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 0.91 (t, 3H, *J* = 7.3 Hz, -CH₃), 1.45 (q, 2H, *J* = 9.4 Hz, -CH₂), 1.65 (q, 2H, *J* = 9.4 Hz, -CH₂), 2.19 (s, 3H, -COCH₃), 2.55-2.66 (m, 1H, Sac-H), 2.87 (d, 1H, *J* = 11.7 Hz, -CH), 3.18-3.40 (m, 6H, Sac-H), 3.44 (s, 2H, Sac-OH), 3.75 (s, 3H, -OCH₃), 4.12 (d, 1H, *J* = 7.2 Hz, Ano-H), 4.52 (t, 1H, *J* = 7.9 Hz, Sac-H), 6.70 (s, 1H, -NH), 6.77 (d, 5H, *J* = 8.4 Hz, Ar-H), 7.06 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.14 (d, 3H, *J* = 6.9 Hz, Ar-H), 7.34 (s, 2H, Ar-OH), 7.63-7.72 (m, 2H, Ar-H), 8.06 (d, 1H, *J* = 9.0 Hz, Ar-H). ¹³C NMR (75 MHz, CDCl₃): δ 207.3, 169.0, 153.3, 152.8, 151.2, 138.8, 136.3, 135.4, 130.2, 129.0, 125.9, 125.4, 124.7, 123.7, 117.0, 114.8, 102.5, 81.4, 80.4, 75.2, 74.3, 70.5, 68.2, 60.0, 55.7, 36.2, 32.0, 30.9, 17.4, 13.9. Elemental Anal. Found: C, 66.87; H, 5.49; N, 1.69. Calc. for C₄₁H₄₁NO₁₂: C, 66.81; H, 5.44; N, 1.62

S1. ¹H NMR spectrum of compound **15**













S7. ¹H NMR spectrum of compound **18**













Optimization of experimental conditions:

The several solvents employed in this optimization are playing key role in the formation of fluorescein based β -aminoglycosyl ketones (15). Initially, the threecomponent Mannich reaction of fluorescein-monoaldehyde (7) with aniline (8) and 1-(4,6-*O*-butylidene- β -D-glucopyranosyl)propan-2-one (14) in the presence of potassium carbonate (K₂CO₃) were conducted and optimization are shown in Table 1. Potassium carbonate (K₂CO₃) catalyzed Mannich reactions in organic solvents such as acetonitrile, THF, 1,2-dichloroethane, methanol, and ethanol gave the desired products **15** as a single isomer without **15a** in low yield with the formation of by-products. We found that a remarkable solvent effect existed in 10 mol% potassium carbonate-catalyzed reaction at reflux temperature. These results showed that methanol was the most suitable solvent for this transformation among others, such as acetonitrile, THF, 1,2-dichloromethane, and ethanol (Table 1, entries 1-5).





^aAll the reactions were carried out using 7 (1.0 mmol), 8 (1.0 mmol), and 14 (1.0 mmol) in 10 mL solvent. ^bIsolated yields.

When the model reaction was carried out under room temperature, reduced yield was observed (Table 1, entry 6). When the reaction was catalyzed by 5 mol%, the reaction time was prolonged to 8 h and the desired product **15** was obtained with only 45% (Table 1, Entry 7). While no conversion of fluorescein based β -aminoglycosyl ketone **15** was

obtained in the absence of potassium carbonate (Table 1, Entry 8). Thus, the most suitable reaction conditions for the formation of **15** were stablished (Table 1, entry 5) as high yield and good *anti* selectivity.





Antioxidant activities of fluorescein based β -aminoglycosylketones (**15-20**) in 96well microplates: (Ac) control; (1) 10 µg; (2) 20 µg; (3) 30 µg; (4) 40 µg; (5) 50 µg; and (6) 60 µg. (a) **15**, (b) **17**, (c) **19**, (d) **18**, (e) **20**, and (f) **16**.

Mass spectrum of compound 15



Mass spectrum of compound 16



Mass spectrum of compound 18



Table 4. DPPH radical scavenging activity of fluorescein based βaminoglycosylketones 15-20

S.No	Concentration	Inhibition of DPPH radical (%)					
	μg/mL	15	16	17	18	19	20
1.	20	11.60	12.58	11.92	13.07	11.42	12.25
2.	40	28.80	31.12	29.13	31.29	31.95	33.6
3.	60	32.25	49.00	46.19	46.68	48.34	45.19
4.	80	44.00	53.97	51.49	52.81	53.31	58.27
5.	100	60.93	66.55	66.55	64.90	61.68	65.56
6.	120	64.90	69.37	68.21	69.70	70.69	71.35