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Synthesis and characterization of novel fluorescent *N*-glycoconjugates

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Abstract—Novel fluorescent *N*-glycoconjugates containing D-glucose, glycine and coumarin or naphthalenetriazole derivatives were prepared by peptide synthesis type methods. The fluorescence properties (spectra, quantum yields) of the compounds were evaluated.

1. Introduction

The glycoconjugates have an enormous potential in drug design.[1] Glycoproteins are widely distributed in nature and the sugar fragment influences their conformation and folding,[2] their properties such as solubility, bioavailability, thermal and proteolytical stability,[3] and enhances the transport through cell membranes.[2,4–6]

Glycopeptides, (which retain the carbohydrate–peptide linkage of a glycoprotein but lack its size and complexity) can mimic natural fragments of glycoproteins and have been largely used as targets for therapeutic agents and as models for biologically relevant systems.[7–11]

Another important field that has registered development is the application of fluorescent labels for several compounds with potential biological activity.[12,13] Among them, the fluorescent peptides[7,14–17] have a large number of applications in biochemistry and biology, namely in studies of protein interactions and conformational analysis.

Fluorescent markers are also being investigated for in vivo imaging studies, for example, in Alzheimer disease.[18] The most used fluorescent markers for peptides are rhodamine, fluorescein, coumarin and their derivatives.[16,19]

Sugars have also been used in the development of fluorescent reagents because they confer water solubility to organic fluorophores with no significant change in absorption and fluorescence properties.[20]

The need for homogeneous samples of the desired glycopeptides leads to significant development of several synthetic strategies.[7,8] The glycoamino acids are the building blocks for glycopeptide synthesis and several routes for their preparation have been reported.[21–25] The most commonly employed methods for the preparation of *N*-glycopeptides proceed through reduction of glycosyl azide to a labile intermediate glycosylamine that is subsequently condensed with the appropriately protected amino acid derivative.[15,21,26]

The work presented in this paper, is part of an ongoing project towards of the synthesis of fluorescent *N*-glycopeptides. Model compounds with a fluorescent aminoacid (the fluorescent labels were introduced at the C-terminus of glycine through an amide bond) were prepared to test the methodologies of synthesis and evaluate the influence either of the amino acid or/and the sugar in the fluorescent properties of the fluorophore (coumarin-3-carboxilic acid and 4-(naphtho[1,2-*d*][1,2,3]triazol-2-yl)benzoic acid). The labeled amino acid will be used in further work as building block for sequence analogues of RGD (arginine-glycine-aspartic acid) motif for binding essays. The absorption and fluorescence properties of these compounds were determined, in acetonitrile, and compared.

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2. Results and discussion

In this work, the preparation of new fluorescent *N*-glycoconjugates based on acetylated D-glucose, glycine and coumarin or naphthalenetriazole derivatives was studied (Scheme 1). Evaluation of the fluorescence properties shown by different substrates was also carried out.

Treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with sodium azide[27] gave the corresponding β-D-glucopyranosyl azide in 86% yield after recrystallisation. Catalytic hydrogenation of the azide[28] with Pd/C afforded the glucosylamine as the β anomer (based on ^{1}H NMR; $J_{H1H2}=8.7$ Hz) in almost quantitative yield (94%) and it was used with no further purification. Compounds **4** and **5** were obtained by the mixed anhydride method in 13 and 42% yield, respectively, but the same method was unsuccessful for the synthesis of compounds **6** and **7**. Therefore, glycine was coupled to the fluorescent dye by the HBTU method (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′,-tetramethyl-uroniumhexa-fluorophosphate) for compound **6** and by the DCC/HOBt (*N*,*N*′-dicyclohexylcarbodiimide/1-hydroxy-benzotriazole) method for **7** with yields of 45%. Hydrolysis of compounds **6** and **7** afforded the corresponding acids **8** and **9** in yields over 80%. Glycoconjugates **10** and **11** were synthesised by DCC/HOBt method in moderate yields, 45 and 46%, respectively. The synthesis of **10** was attempted by the Staudinger reaction between glycosyl azide and amino acid-dye substrate **8** in the presence of tributyl phosphine.[29] This method afforded the α-anomer, as deduced by ^{1}H NMR spectrum ($J=3.6$ Hz for the anomeric proton), instead of the β-anomer obtained by the DCC/HOBt method.

The compounds were isolated and characterized by NMR spectroscopy (^{1}H, ^{13}C, HMQC, HMBC) and elemental analysis or HRMS.

As the compounds prepared differ markedly in water solubility, acetonitrile, a common polar aprotic solvent, was chosen to measure their spectral properties. Besides, acetonitrile avoids changes in spectral curves resulting from dissociation of carboxylic hydrogen in non conjugated probes. Then it was possible to compare a substitution effect on fluorescence properties of starting dyes and their conjugates.

The spectra for compounds **2** and **10** and **3** and **11** are shown on Figures 1 and 2, respectively.

UV–vis absorption spectrum of **2** shows vibrational structure at 340 ($\varepsilon_{max}\approx 24{,}500$ dm^3 mol^{-1} cm^{-1}) and 360 nm, while for compound **3**, only a broad band at 300 nm ($\varepsilon_{max}\approx 11{,}400$ dm^3 mol^{-1} cm^{-1}) and a shoulder around 330 nm are observed. Replacement of the carboxyl group by amido groups (such as in **4**, **6** and **10**) has no significant effect on the position or molar absorptivity of the long-wavelength absorption bands (Fig. 1). A similar observation may be made for the position of the long-wavelength absorption bands of **3**, **5**, **7** and **11** (Fig. 2). However, the carboxylic acid **3** has a lower molar absorptivity ($\varepsilon_{max}\approx 11{,}400$ dm^3 mol^{-1} cm^{-1}) than the corresponding amides ($\varepsilon_{max}\approx 13{,}200$ dm^3 mol^{-1} cm^{-1}).

The steady-state fluorescence spectrum of **2** shows an emission band also with vibrational structure (maxima at 370 and 380 nm). The transformation of the carboxyl group of **2** to amido groups (**4**, **6** and **10**) does not affect either the shape or position of the fluorescence band but it has considerable effect on fluorescence quantum yield (53% for **2** and close to 100% for the other compounds, standard deviation is $\leq$2%). The relatively high absorption coefficient of the first absorption band of **2** and high fluorescence quantum yield lead to the conclusion that the first absorption band of the studied triazole derivatives has the character of S_0–$S_1(\pi\pi^*)$ transition. Moreover, the high quantum yield shows that none of possible triplet $n\pi^*$ states (nonbonding electrons on two heterocyclic nitrogens and nonbonding electrons on carbonyl group) is situated below $S_1(\pi\pi^*)$ state.

The steady-state fluorescence spectrum of **3** displays a broad emission band with maximum at 400 nm (shoulder at 370 nm) similar to the emission spectra of derivatives **5**, **7** and **11**. Fluorescence quantum yields were 1.8% (standard deviation is 0.1%) for **3** and 2.1–2.4% for compounds **5**, **7** and **11**.

As the spectra of compounds **2**, **4**, **6** and **10** and **3**, **5**,**7** and **11** show the same patterns, it is possible to conclude that the substitution on carboxylic groups of both tested fluorescence probes does not affect the π-electronic structure of fluorophores. Therefore, character, energy and sequence of excited states do not change.

3. Conclusions

The fluorescent probes studied show that the transformation of the carboxylic group into amido group in the different *N*-conjugates does not affect either the shape or the position of their fluorescence bands. On the other hand, in case of naphthalenetriazole derivatives, it has considerable effect on fluorescence quantum yield ~50% and almost 100% for the original carboxylic acid and amides, respectively. No appreciable changes in low quantum fluorescence yields (2%) were detected among original coumarin-3-carboxylic acid and its amido *N*-conjugates.

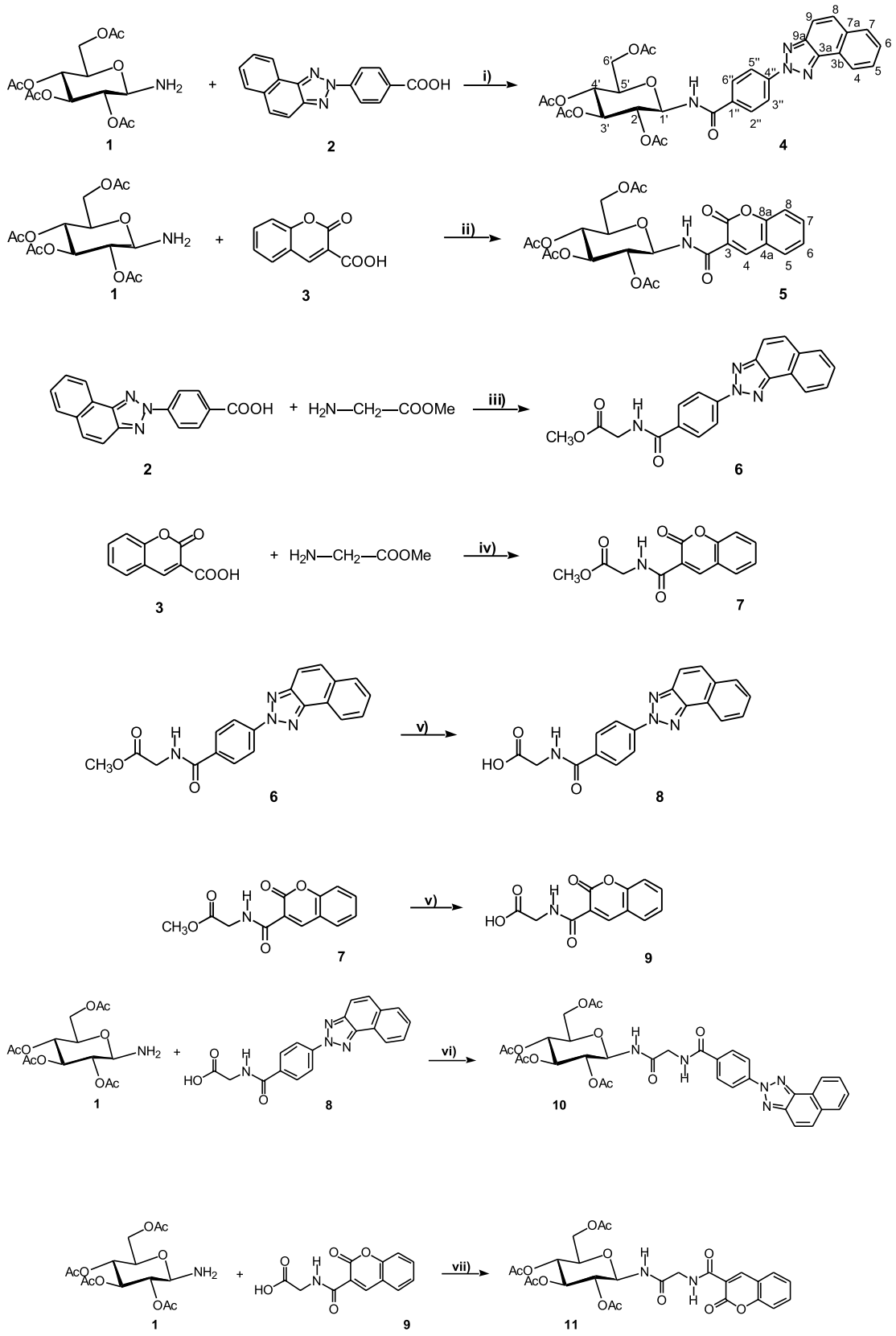
Our findings showed that the usual methods used in peptide synthesis gave acceptable results for the coupling of the glycosylamine to a fluorescent carboxylic dye or to fluorescent amino acid.

This methodology may provide a useful contribution for the design of new fluorescent glycopeptides.

4. Experimental

4.1. General

Dry solvents, referred to in the ensuing experiments, were prepared as follows: Acetone was refluxed over magnesium sulphate, decanted, stirred overnight with calcium chloride, decanted, refluxed over fresh magnesium sulphate, distilled from it and stored over 4 Å molecular sieves;



Scheme 1. Reagents and conditions: (i) $ClCO_2Et$, Et_3N, DMF, rt, 48 h; (ii) $ClCO_2Et$, Et_3N, acetone, reflux, 3 h; (iii) HBTU, DIPEA, DMF, rt, 12 h; (iv) DCC/HOBt, Et_3N, EtOAc, rt, 12 h; (v) (1) NaOH, MeOH, rt, 4 h; (2) HCl; (vi) DCC/HOBt, DMF, rt, 12 h; (vii) DCC/HOBt, DMF, rt, 48 h.

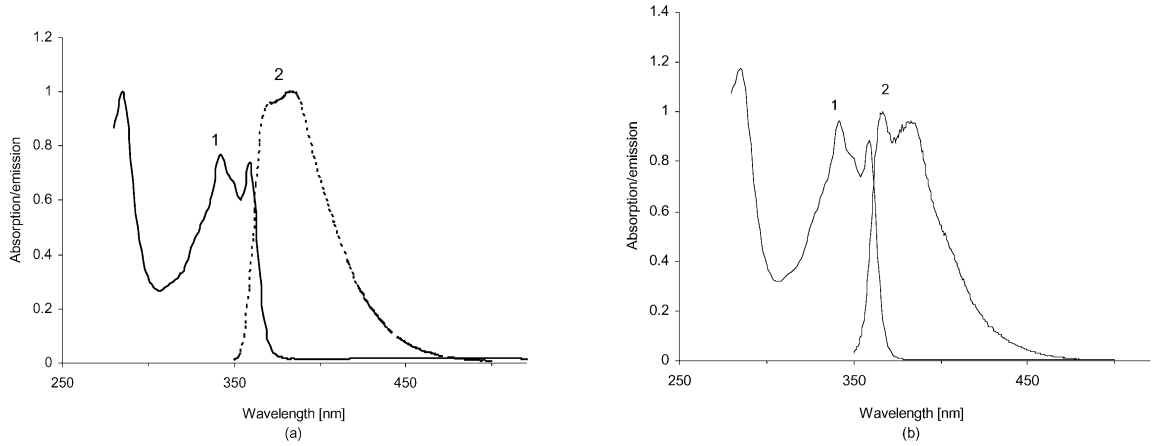


Figure 1. Normalised absorption (curve 1) and fluorescence emission (curve 2) spectra of naphthalenetriazole derivative **2** (a) and **10** (b).

dichloromethane was refluxed over phosphorus pentoxide, distilled from it and stored over 4 Å molecular sieves. Light petroleum refers to the fraction boiling in the range 40–60 °C. Sodium azide was dried in vacuo overnight.

The reactions were followed by thin layer chromatography, which was carried out on pre-coated plates (Merck Kieselgel 60F_{254}) and compounds were visualized by UV-light and exposure to vaporized iodine. Merck Kieselgel 60 (230–400 mesh) was used in column chromatography. Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. Specific optical rotations were calculated from measurements carried out with an Optical Activity-AA-1000 Polarimeter at 27 °C. IR spectra were recorded on a FTIR/Diffus Bomem MB spectrometer. ^{1}H and ^{13}C NMR data were recorded on a Varian Unity Plus 300 Spectrometer in $CDCl_3$ and DMSO-d_6; δ ppm were measured versus residual peak of the solvent and J values are given in Hz. HMQC and HMBC experiments were carried out for complete assignment of proton and carbon signals in the NMR spectra (in descripton of ^{1}H signals app means apparent). Elemental analyses were carried out on a Leco CHNS 932 instrument. MS spectra were obtained in a VG Autospec mass spectrometer. Absorption spectra were recorded using a Perkin-Elmer Lambda 35 spectrophotometer. Steady-state fluorescence spectra were measured on a Perkin-Elmer LS-5 spectrofluorometer. Quantum yields were determined in acetonitrile using quinine sulphate as the fluorescence standard. The path length was 1 cm in quartz cells.

4.1.1. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylazide.[26] Dry sodium azide (0.163 g, 2.5 mmol) was added to a solution of α-acetobromoglucose (0.411 g, 1.0 mmol) in dry acetone (40 mL) and the mixture refluxed for 7 h. Then it was portioned between dichloromethane (40 mL) and cold water (30 mL). The aqueous phase was extracted with dichloromethane, dried ($MgSO_4$) and evaporated affording the impure azide as a light yellow solid in quantitative yield. Recrystallisation from dichloromethane/diethyl ether/light petroleum afforded the azide as a crystalline white solid in 86% yield, mp 125.6–126.5 °C (lit.[30] 126–127 °C). $[\alpha]_D$ −29 (c 2.0, $CHCl_3$); ν_{max} (Nujol) 2115; 1746; 1213; 1105–1042 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.02, 2.04, 2.09 and 2.11 (4s, 12H, 4×OCH_3), 3.80 (ddd, J=10.0, 4.8, 2.4 Hz, 1H, H-5), 4.18 (dd, J=12.6, 2.4 Hz, 1H, H_a-6), 4.29 (dd, J=12.6, 4.8 Hz, 1H, H_b-6), 4.66 (d, J=9.0 Hz, 1H, H-1), 4.98 (app t, J=9.3, 9.6 Hz, 1H, H-2), 5.11 (app t, J=9.6 Hz, 1H, H-4), 5.23 (app t, J=9.3, 9.6 Hz, 1H, H-3).

4.1.2. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylamine[28] **(1).** To a solution of the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (0.336 g, 0.9 mmol) in ethyl acetate/methanol 1:1 (30 mL), Pd/C 5% (0.119 g) was added. The

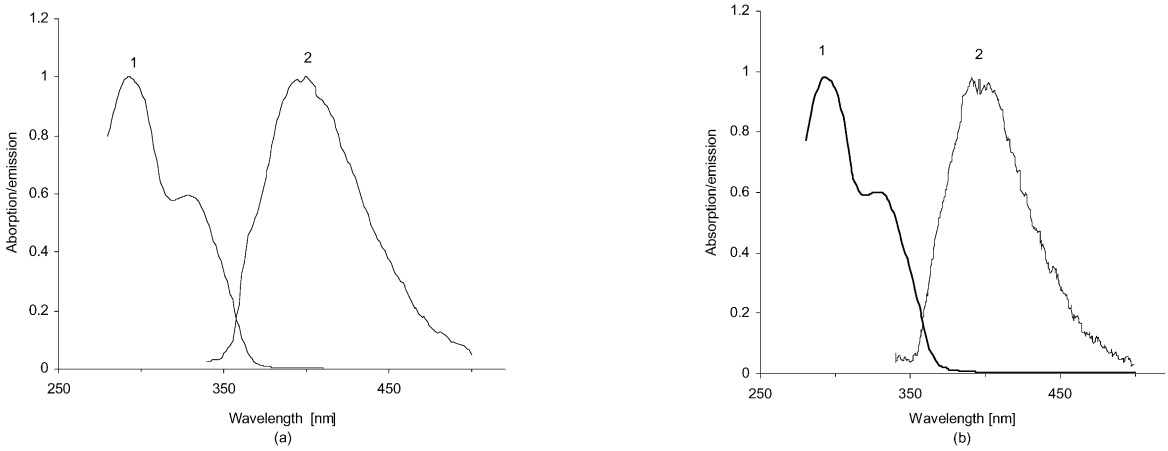


Figure 2. Normalised absorption (curve 1) and fluorescence emission (curve 2) spectra of coumarin derivatives **3** (a) and **11** (b).

resulting mixture was deaerated for 30 min under a nitrogen stream. Then it was submitted to a hydrogen atmosphere, at normal pressure, for 3 h. The final mixture was filtered through a pad of Celite and concentrated affording the product as a yellowish solid in 94% that was used with no further purification, mp 105–106 °C, mp 115–116 °C after crystallization from methanol/light petroleum (lit.,[31] mp not reported, unstable, mp[32] 138–140 °C). $[\alpha]_D+16$ (*c* 2.0, $CHCl_3$); ν_{max} (Nujol) 3410; 3337; 2725; 2668; 2257; 1754; 1639; 1377; 1224; 1095–1062 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.02, 2.04, 2.08 and 2.10 (4s, 12H, $4\times OCH_3$), 2.02–2.10 (br s, 2H, NH_2), 3.70 (ddd, $J=10.0$, 4.8, 2.4 Hz, 1H, H-5), 4.12 (dd, $J=12.3$, 2.4 Hz, 1H, H_a-6), 4.20 (d, $J=8.7$ Hz, 1H, H-1), 4.24 (dd, $J=12.3$, 4.8 Hz, 1H, H_b-6), 4.84 (app t, $J=9.3$, 9.6 Hz, 1H, H-2), 5.05 (app t, $J=9.6$ Hz, 1H, H-4), 5.25 (app t, $J=9.3$, 9.6 Hz, 1H, H-3). Anal. Calcd for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.10; N, 4.03. Found: C, 48.44; H, 6.09; N, 4.07.

4.1.3. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzamide (4). To a solution of compound **2** (0.280 g, 0.969 mmol) in DMF (200 mL) at −5 °C, triethylamine (0.098 g, 0.134 mL, 0.969 mmol) and ethyl chloroformate (0.108 g, 0.096 mL, 0.969 mmol) were added. The solid precipitated was filtered off and the filtrate was maintained at −5 °C. Then, it was added to the amine (**1**) (0.337 g, 0.969 mmol). The resulting mixture was stirred at rt for 48 h. After evaporation, the crude residue (0.404 g) was purified by flash column chromatography using diethyl ether/light petroleum 2:1–2.5:1 as eluent. The isolated fraction (0.078 g, 13% yield) was recrystallised from ethyl acetate/light petroleum affording the amide **4** as a light pink solid, mp 230–233 °C (dec). $[\alpha]_D-53$ (*c* 1.0, $CHCl_3$); ν_{max} (Nujol) 3430; 1748; 1680; 1607; 1370; 1291; 1228; 1217 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.07, 2.08, 2.09 and 2.11 (4s, 12H, $4\times OCH_3$), 3.95 (ddd, $J=10.0$, 4.0, 1.8 Hz, 1H, H-5′), 4.15 (dd, $J=12.6$, 1.8 Hz, 1H, H_a-6′), 4.40 (dd, $J=12.6$, 4.0 Hz, 1H, H_b-6′), 5.11 (app t, $J=9.6$, 9.9 Hz, 1H, H-2′ or H-4′), 5.15 (app t, $J=9.3$, 10.2 Hz, 1H, H-2′ or H-4′), 5.45 (app t, $J=9.9$, 9.6 Hz, 1H, H-3′), 5.48 (t, $J=9.0$ Hz, 1H, H-1′), 7.16 (d, $J=9.0$ Hz, 1H, NH), 7.62–7.82 (m, 4H, H-5, H-6, H-9, H-8), 7.92 (dd, $J=8.1$, 1.8 Hz, 1H, H-4), 7.98 (d, $J=9.0$ Hz, 2H, H-3″ and H-5″), 8.50 (d, $J=9.0$ Hz, 2H, H-2″ and H-6″), 8.65 (dd, $J=7.5$, 1.8 Hz, 1H, H-7). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.77, 20.74, 20.60 ($4\times CH_3$), 61.58 (C-6′), 68.16 (C-2′ or C-4′), 70.87 (C-2′ or C-4′), 72.51 (C-3′), 73.65 (C-5′), 79.03 (C-1′), 116.31 (C-8 or C-9), 119.96 (C-2″ and C-6″), 123.40 (C-7), 124.77 (C-3b), 127.73 (C-5), 127.92 (C-6), 128.70 (C-3″and C-5″), 129.00 (C-4), 130.42 (C-8 or C-9), 131.95 (C-4″), 132.51 (C-7a), 143.03 (C-1″), 143.42 (C-3a), 144.03 (C-9a), 166.10 (C=O from dye), 169.60, 169.88, 170.64 and 171.74 ($4\times$C=O, acetyl). HRMS (FAB) calcd for $C_{31}H_{30}N_4O_{10}$ ($M^+ +1$) 619.2040, found 619.2051.

4.1.4. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(2-oxo-2*H*-chromene)-3-carboxamide (5). To a solution of compound **3** (0.138 g, 0.723 mmol) in dry acetone (20 mL) at −5 °C, triethylamine (0.146 g, 0.200 mL, 1.45 mmol) and ethyl chloroformate (0.157 g, 0.143 mL, 1.45 mmol) were added. The solid precipitated was filtered off and the filtrate was maintained at −5 °C. Then, it was added to the amine (**1**) (0.251 g, 0.723 mmol) and the resulting mixture was refluxed for 3 h and evaporated. The crude residue (0.357 g) was purified by flash column chromatography using ethyl acetate/diethyl ether 1:5 as eluent. The isolated fraction (0.158 g, 42% yield) was recrystallised from dichloromethane/diethyl ether/light petroleum affording the amide **5** as a white solid, mp 160.0–162.3 °C. $[\alpha]_D-26$ (*c* 2.0, $CHCl_3$); ν_{max} (Nujol) 3306; 2725; 2670; 1748; 1722; 1672; 1610; 1376; 1214; 1159–1034 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.01, 2.04, 2.05 and 2.10 (4s, 12H, $4\times OCH_3$), 3.90 (ddd, $J=10.0$, 4.0, 2.1 Hz, 1H, H-5′), 4.14 (dd, $J=12.6$, 2.1 Hz, 1H, H_a-6′), 4.30 (dd, $J=12.6$, 4.0 Hz, 1H, H_b-6′), 5.17 (app q, $J=9.6$, 9.9, 9.6 Hz, 2H, H-2′ and H-4′), 5.36 (app t, $J=9.3$, 9.6 Hz, 1H, H-3′), 5.50 (app t, $J=9.0$, 9.3 Hz, 1H, H-1′), 7.38–7.44 (m, 2H, H-8 and H-7), 7.68–7.73 (m, 2H, H-6 and H-5), 8.90 (s, 1H, 4-H), 9.31 (d, $J=9.6$ Hz, 1H, NH). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.47, 20.54, 20.56 and 20.69 ($4\times CH_3$), 61.27 (C-6′), 68.04 (C-2′ or C-4′), 70.30 (C-2′ or C-4′), 73.01 (C-3′), 73.63 (C-5′), 78.16 (C-1′), 116.75 (C-7), 117.43 (C-3), 118.28 (C-8a), 125.36 (C-8), 129.95 (C-5), 134.65 (C-6), 149.48 (C-4), 154.62 (C-4a), 160.76 (C=O, coumarin), 162.25 (C=O, amide), 169.39, 169.73, 170.03 and 170.65 ($4\times$C=O, acetyl). Anal. Calcd for $C_{24}H_{25}NO_{12}$: C, 55.48; H, 4.86; N, 2.70. Found: C, 55.47; H, 5.08; N, 2.56. FAB ($M^+ +1$) 520.22.

4.1.5. 4-Naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino acetic acid methyl ester (6). To a solution of **2** (0.145 g, 0.5 mmol), HGlyOMe, HCl (0.063 g, 0.5 mmol) and DIPEA (0.170 mL, 1 mmol) in DMF (10 mL), cooled to +5 °C, HBTU (0.190 g, 0.5 mmol) was added. The reaction mixture was stirred at rt overnight and diluted with water (50 mL). After cooling for 3 h the solid precipitated was filtered and washed with water. The crude solid was purified by column chromatography using ethyl acetate/dichloromethane 5:1 as eluent. Recrystallisation from methanol/dichloromethane/light petroleum afforded the pure compound as an orange solid in 45% yield, mp 229–231 °C. $[\alpha]_D-2$ (*c* 1.0, DMF); ν_{max} (KBr) 3362; 1754; 1643; 1606; 1367; 1295; 1206; 1179 cm^{-1}. ^1H NMR (DMSO-d_6) δ (ppm): 3.67 (s, 3H, OCH_3), 4.06 (d, $J=5.7$ Hz, 2H, CH_2), 7.69–7.79 (m, 2H, H-5 and H-6), 7.91 (s, 2H, H-8 and H-9), 8.07 (dd, $J=6.9$, 2.0 Hz, 1H, H-4), 8.15 (d, $J=8.7$ Hz, 2H, H-2″ and H-6″), 8.42 (d, $J=8.7$ Hz, 2H, H-3″and H-5″), 8.55 (dd, $J=7.2$, 2.0 Hz, 1H, H-7), 9.19 (t, $J=5.7$ Hz, 1H, NH). ^{13}C NMR (DMSO-d_6) δ (ppm): 41.35 (CH_2), 51.87 (OCH_3), 116.27 (C-8 or C-9), 119.46 (C-3″ and C-5″), 122.88 (C-7), 123.87 (C-3b), 128.18 (C-5), 128.30 (C-6), 129.18 (C-2″ and C-6″), 129.39 (C-4), 130.55 (C-8 or C-9), 132.17 (C-7a), 133.38 (C-4″), 141.49 (C-1″), 142.53 (C-3a), 143.40 (C-9a), 165.66 (C=O, amide), 170.38 (C=O, ester). Anal. Calcd for $C_{20}H_{16}N_5O_3$: C, 66.66; H, 4.48; N, 15.55. Found: C, 66.25; H, 4.57; N, 15.03. FAB ($M^+ +1$) 360.15.

4.1.6. [(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-acetic acid methyl ester (7). To a solution of **3** (1.90 g, 10 mmol) in ethyl acetate (100 mL) HOBt (1.70 g, 10 mmol) was added. After cooling to 0 °C, DCC (2.16 g, 10.5 mmol) was added followed by addition of the HGlyOMe, HCl (1.26 g, 10 mmol) and triethylamine (1.4 mL, 10 mmol). The reaction mixture was stirred at rt overnight and the insoluble materials were filtered off. The

organic layer was successively washed with 5% $NaHCO_3$, water, 5% citric acid, water and dried. The solvent was removed under reduced pressure and the resulting product (1.17 g, 45%) crystallized from ethyl acetate/light petroleum, mp 184.8–187.4 °C. $[\alpha]_D$ −2 (*c* 1.0, DMF); ν_{max} (KBr) 3332; 1748; 1713; 1607; 1372; 1294; 1218 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 3.81 (s, 3H, OCH_3), 4.27 (d, J= 6.0 Hz, 2H, CH_2), 7.37–7.44 (m, 2H, H-8 and H-6), 7.67–7.72 (m, 2H, H-7and H-5), 8.92 (s, 1H, 4-H), 9.26 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ (ppm): 41.62 (CH_2), 52.35 (OCH_3), 116.64 (C-8), 117.90 (C-3), 118.44 (C-8a), 125.28 (C-6), 129.84 (C-5), 134.23 (C-7), 148.69 (C-4), 154.48 (C-4a), 161.16 (C=O, coumarin), 161.83 (C=O, amide), 169.67 (C=O, ester). Anal. calcd for $C_{13}H_{11}NO_5$: C, 59.76; H, 4.25; N, 5.36. Found: C, 59.93; H, 4.46; N, 5.18. FAB (M^+ + 1) 262.05.

4.1.7. 4-Naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino acetic acid (8). The ester **6** (0.268 g, 0.744 mmol) was dissolved in methanol (1 mL) and 1 M NaOH (1.9 mL, 1.861 mmol) added. The mixture was stirred at rt for 4 h and 1 M HCl (0.744 mL, 0.744 mmol) was added. The methanol was removed under reduced pressure and the residue thus, obtained cooled in an ice bath and acidified to pH ~ 2–3 with 1 M HCl with vigorous stirring for 1 h. The solid precipitated was filtered, washed with water and dried. Compound **8** was isolated in 87% yield and it was used without further purification, mp 209–213 °C. $[\alpha]_D$ −4 (*c* 1.0, DMF); ν_{max} (KBr) 3403; 2554; 1941; 1681; 1633; 1605; 1366; 1311; 1289; 1228; 1167 cm^{-1}. ^1H NMR (DMSO-d_6) δ (ppm): 3.97 (d, J=5.7 Hz, 2H, CH_2), 7.68–7.80 (m, 2H, H-5 and H-6), 7.92 (s, 2H, H-8 and H-9), 8.08 (br d, J= 7.5 Hz, 1H, H-4), 8.15 (d, J=8.4 Hz, 1H, H-2″ or H-6″), 8.20 (d, J=8.7 Hz, 1H, H-2″ or H-6″), 8.42 (d, J=8.7 Hz, 2H, H-3″ and H-5″), 8.55 (br d, J=7.2 Hz, 1H, H-7), 9.05 (t, J=5.7 Hz, 1H, NH), 12.80 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 41.34 (CH_2), 116.35 (C-8 or C-9), 119.59 (C-3″ or C-5″), 119.70 (C-3″ or C-5″), 123.02 (C-7), 123.98 (C-3b), 128.34 (C-5), 128.44 (C-6), 129.25 (C-2″ or C-6″), 129.50 (C-4), 130.68 (C-9 or C-8), 131.34 (C-2″ or C-6″), 132.29 (C-7a), 133.70 (C-4″), 141.57 (C-1″), 142.66 (C-3a), 143.52 (C-9a), 165.77 (C=O, amide), 171.32 (C=O, acid). HRMS (EI) calcd for $C_{19}H_{14}N_4O_3$ 346.1066, found 346.1062.

4.1.8. [(2-Oxo-2*H*-chromene-3-carbonyl)-amino]-acetic acid (9). The ester **7** (0.769 g, 3 mmol) was dissolved in methanol (5 mL) and 1 M NaOH (7.5 mL, 7.5 mmol) added. The mixture was stirred at rt for 4 h and 1 M HCl (3.0 mL, 3 mmol) was added. The methanol was removed under reduced pressure and the residue thus, obtained cooled in an ice bath and acidified to pH ~ 2–3 with 1 M HCl with vigorous stirring for 1 h. The solid precipitated was filtered, washed with water and dried. Compound **9** was isolated in 86% yield and it was used without further purification, mp 238–239 °C (dec). $[\alpha]_D$ −4 (*c* 1.0, DMF); ν_{max} (KBr) 3315; 2683; 1760; 1712; 1634; 1608; 1372; 1294; 1228; 1162 cm^{-1}. ^1H NMR (DMSO-d_6) δ (ppm): 4.05 (d, J= 5.7 Hz, 2H, CH_2), 7.44 (t, J=7.4 Hz, 1H, H-6), 7.51 (d, J= 8.4 Hz, 1H, H-8), 7.75 (dt, J=1.5, 8.0 Hz, 1H, H-7), 7.99 (dd, J=1.5, 7.8 Hz, 1H, H-5), 8.90 (s, 1H, H-4), 9.03 (t, J= 5.7 Hz, 1H, NH), 12.8 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 41.52 (CH_2), 116.18 (C-8), 118.19 (C-3), 118.43 (C-8a), 125.19 (C-6), 130.43 (C-5), 134.32 (C-7), 148.12 (C-4), 154.00 (C-4a), 160.39 (C=O, coumarin), 161.18 (C=O, amide), 170.81 (C=O, acid). Anal. Calcd for $C_{12}H_9NO_5$: C, 58.18; H, 3.66; N, 5.65. Found: C, 58.10; H, 3.93; N, 5.60.

4.1.9. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino acetamide (10). To a solution of **8** (0.258 g, 0.744 mmol) in DMF (10 mL), glycosylamine **1** (0.258 g, 0.744 mmol) and HOBt (0.111 g, 0.818 mmol) were added. After cooling to +5 °C, DCC (0.161 g, 0.781 mmol) was added and the resulting mixture stirred at rt overnight. Then, water (50 mL) was added and the mixture cooled for 3 h. The solid precipitated was filtered, dried (0.414 g, 82%) and purified by flash column chromatography using ethyl acetate/dichloromethane 5:1 as eluent. The isolated fraction (0.226 g, 45%) was recrystallised from dichloromethane/light petroleum, affording pure **10** as an orange solid, mp 232.2–233.0 °C. $[\alpha]_D$ +5 (*c* 2.0, $CHCl_3$); ν_{max} (neat) 3413; 1745; 1607; 1370; 1227; 1160 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 2.01, 2.03, 2.07 and 2.09 (4s, 12H, 4×OCH_3), 3.90 (ddd, J=12.6, 4.0, 2.1 Hz, 1H, H-5′), 4.12–4.19 (m, 3H, H_a-6′ and CH_2Gly), 4.31 (dd, J=12.6, 4.0 Hz, H_b-6′), 5.02 (t, J=9.6 Hz, 1H, H-4′), 5.10 (app t, J=9.6, 10.0 Hz, 1H, H-2′), 5.34 (app t, J=9.3, 9.6 Hz, 2H, H-1′ and H-3′), 7.30 (t, J=5.1 Hz, 1H, NHGly), 7.51 (d, J=9.0 Hz, 1H, NH), 7.82 (app d, 1H, J=7.4 Hz, H-4), 8.00 (d, J=8.7 Hz, 2H, H-2″ and H-6″), 8.38 (d, J=8.7 Hz, 2H, H-3″ and H-5″), 8.53 (app d, 1H, J=8.0 Hz, H-7), 7.60–7.70 (m, 4H, H-5, H-6, H-8 and H-9). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.72, 20.60, 20.53 (4×CH_3), 43.74 (CH_2Gly), 61.58 (C-6′), 68.05 (C-2′ or C-4′), 70.34 (C-2′ or C-4′), 72.73 (C-3′), 73.63 (C-5′), 78.21 (C-1′), 116.17 (C-8 or C-9), 119.67 (C-3″and C-5″), 123.28 (C-7), 124.65 (C-3b), 127.58 (C-5), 127.74 (C-6), 128.57 (C-2″ and C-6″), 128.87 (C-4), 130.20 (C-8 or C-9), 132.36 (C-7a), 132.44 (C-4″), 142.55 (C-1″), 143.18 (C-3a), 143.80 (C-9a), 166.85 (C=O from dye), 169.52 (C=O, acetyl), 169.91 (C=O, acetyl), 169.97 (C=O, aminoacid), 170.72 (C=O, acetyl), 170.94 (C=O, acetyl). Anal. Calcd for $C_{33}H_{33}N_5O_{11}$: C, 58.66; H, 4.92; N, 10.37. Found: C, 58.84; H, 5.06; N, 10.24.

4.1.10. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-[(2-oxo-2*H*-chromene-3-carbonyl)-amino]-acetamide (11). To a solution of **9** (0.1236 g, 0.5 mmol) in DMF (10 mL), glycosylamine **1** (0.1737 g, 0.5 mmol) and HOBt (0.086 g, 0.55 mmol) were added. After cooling to +5 °C, DCC (0.108 g, 0.525 mmol) was added and the resulting mixture stirred at rt for 48 h. Then, water (50 mL) was added and the mixture cooled for 3 h. The solid precipitated was filtered, dried (0.131 g, 46%) and purified by flash column chromatography using ethyl acetate as eluent. The isolated fraction (0.067 g, 23%) was recrystallised from dichloromethane/light petroleum, affording pure **11** as a white solid, mp 245–246 °C. $[\alpha]_D$ −11 (*c* 2.0, $CHCl_3$); ν_{max} (neat) 3413; 1746; 1610; 1370; 1229 cm^{-1}. ^1H NMR ($CDCl_3$) δ (ppm): 1.99, 2.03 and 2.09 (3s, 12H, 4×OCH_3), 3.84 (ddd, J=12.6, 4.5, 2.4 Hz, 1H, H-5′), 4.08–4.15 (m, 1H, Ha-6′), 4.14 (d, J=5.7 Hz, 1H, CH_2Gly), 4.30 (dd, J= 12.6, 4.5 Hz, 1H, Hb-6′), 4.90 (t, J=9.6 Hz, 1H, H-4′), 5.05 (app t, J=9.6, 10.0 Hz, 1H, H-2′), 5.24 (app t, J=9.3, 9.6 Hz, 1H, H-1′), 5.30 (t, J=9.6 Hz, 1H, H-3′), 7.01 (d,

J=9.3 Hz, 1H, NH), 7.40 (d, J=7.8 Hz, 1H, H-8), 7.44 (d, J=7.8 Hz, 1H, H-5), 7.71 (t, J=7.8 Hz, 2H, H-6 and H-7), 8.91 (s, 1H, H-4), 9.26 (t, J=4.8 Hz, 1H, NHGly). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.50, 20.55 and 20.70 (4×CH_3), 43.89 (CH_2Gly), 61.58 (C-6′), 68.09 (C-2′ or C-4′), 70.26 (C-2′ or C-4′), 72.55 (C-3′), 73.56 (C-5′), 78.22 (C-1′), 116.76 (C-7), 117.79 (C-3), 118.44 (C-8a), 125.41 (C-8), 129.92 (C-5), 134.44 (C-6), 148.85 (C-4), 154.54 (C-4a), 161.30 (C=O, coumarin), 162.62 (C=O, coumarin amide), 169.28 (C=O, aminoacid), 169.50, 169.80, 170.63 and 171.10 (4×C=O, acetyl). Anal. Calcd for $C_{26}H_{28}N_2O_{13}$: C, 54.16; H, 4.90; N, 4.86. Found: C, 53.85; H, 5.19; N, 4.72. HRMS (FAB) calcd for $C_{26}H_{29}N_2O_{13}$ (M^++1) 577.1670, found 577.1671.

4.1.11. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-naphtho[1,2-*d*][1,2,3]triazol-2-yl-benzoylamino-acetamide (10) (α-anomer). To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (0.195 g, 0.52 mmol), in anhydrous CH_2Cl_2 (20 mL), under a stream of argon, compound **8** (0.20 g, 0.58 mmol) was added. The mixture was cooled to −78 °C, stirred for 10 min followed by the addition of tributylphosphine (0.129 mL, 0.522 mmol). After 10 min, the stream of argon was cut off and the reaction mixture was stirred at −78 °C for 8 h and at rt overnight. Then, it was kept in the refrigerator for 3 h. The precipitate (tributylphosphine oxide) was filtered off and the solution concentrated affording an oily residue, which was purified by column chromatography (eluent: methanol/dichloromethane 3:97). The isolated fraction (0.307 g) was further purified by preparative layer chromatography, using as eluents, successively, diethyl ether/dichloromethane 1:1 (two runs) and dichloromethane (three runs). The required band was removed and extracted with dichloromethane. The solvent was evaporated yielding a white solid (0.024 g, 7%, contaminated with traces of the β-anomer), mp 228.7–230.4 °C; ν_{max} (neat) 3413; 1745; 1607; 1370; 1227; 1160 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ (ppm): 2.07, 2.10, 2.11 and 2.12 (4s, 12H, 4×OCH_3), 4.21–4.77 (m, 7H, CH_2 Gly, 2×H-6′, H-5′, H-4′and H-3′), 5.24 (app d, J=4.2 Hz, 1H, H-2′), 5.57 (d, J=3.6 Hz, 1H, H-1′), 6.78 (t, J=4.8 Hz, 1H, NHGly), 7.63–7.81 (m, 5H, H-9, H-8, H-6, H-5, NH), 7.91 (dd, J=7.5, 1.5 Hz, 1H, H-4), 8.03 (d, J=9.0 Hz, 2H, H-2″ and H-6″), 8.49 (d, J=8.7 Hz, 2H, H-3″ and H-5″), 8.65 (dd, J=7.8, 1.8 Hz, 1H, H-7). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.48, 20.71, 20.75 (4×CH_3), 41.99 (CH_2Gly), 60.94 (C-3′ and C-4′), 61.80 (C-6′), 66.32 (C-1′), 67.46 (C-2′), 74.12 (C-5′), 116.32 (C-5 or C-6 or C-8 or C-9), 119.88 (C-3″ and C-5″), 123.40 (C-7), 124.82 (C-3b), 127.70 (C-5 or C-6 or C-8 or C-9), 127.86 (C-5 or C-6 or C-8 or C-9), 128.51 (C-2″ and C-6″), 129.00 (C-4), 130.33 (C-5 or C-6 or C-8 or C-9), 132.50 (C-7a or C-4″), 133.06 (C-7a or C-4″), 142.64 (C-1″), 143.35 (C-3a), 143.96 (C-9a), 169.48 (C=O from dye), 169.56, 170.05 and 170.12 (C=O, acetyl), 170.49 (C=O, aminoacid).

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