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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;