Glycoconjugates Based on Supramolecular Tröger’s Base Scaffold: Synthesis, Photophysics, Docking, and BSA Association Study

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ABSTRACT: This study presents new Tröger’s bases bearing glycosyl moieties obtained from a copper-catalyzed azide−alkyne cycloaddition reaction. The Tröger’s bases present absorption maxima close to 275 nm related to fully spin and symmetry-allowed electronic transitions. The main fluorescence emission located at 350 nm was observed with no influence on the glycosyl moieties. Furthermore, protein detection studies have been performed using bovine serum albumin (BSA) as a model protein, and results have shown a strong interaction between some of the compounds through a static fluorescence suppression mechanism related to the formation of a glycoconjugate−BSA complex favored by the glycosyl subunit. Moreover, docking was also studied for better understanding the suppression mechanism and indicated that the glycosyl and triazole moieties increase the affinity with BSA.

INTRODUCTION

Tröger’s bases are C₂-symmetric chiral tetracyclic molecules, bearing a methano-1,5-diazocine ring system, disposed in a unique V-shaped twisted structure. In this conformation, the two aryl rings are almost at a 90° dihedral angle, as presented in Figure 1 for 2,8-bis(methyl)-6H,12H-(5,11)-methanodibenzo[b,f][1,5]diazocine (1).

These molecules have been known for more than a century, since the first report from Tröger.† Thereafter, a number of applications in supramolecular chemistry have been proposed. In this sense, the pioneering work from Wilcox‡ reported the use of Tröger’s bases in molecular recognition studies as a torsional molecular balance to quantify weak interactions such as head−tail and CH−π, which have important implications for the folding of proteins.3 Since these initial reports, different designs have been presented.3−11 For example, these molecules have been described as synthetic receptors for carboxylic acids,12 terpenes,13 adenine and biotin,14 monovalent cations,15 optical sensing,16−18 and dye-sensitized solar cell (DSSC) applications.19 Similarly, polyaromatic arylated Tröger’s bases20 and derivatives bearing heterocyclic ring systems such as phenanthroline,21 naphthalimide,22 and acridine23 derivatives have been studied in DNA intercalation. Additional applications include chiral solvating agents,24 catalysts,25 ligands in metal complexes,26 new materials,27 and compounds with biological activity.28 On the other hand, Tröger’s bases bearing carbohydrates in their structure have not received much attention in the literature, despite the interesting properties that might arise from the introduction of a sugar moiety into the supramolecular structure.29,30 Carbohydrates are naturally available and therefore usually abundant and inexpensive.31 The polyoxygenated scaffold of...
carbohydrates is a valuable source of functionality that has been used to introduce a complexation or recognition site in a supramolecular structure for diverse applications. In this sense, different chemosensors for the detection of metals such as nickel(II),\(^{32,33}\) copper(II),\(^{34,35}\) zinc(II),\(^{36}\) silver(I),\(^{37}\) and protein-responsive electrochemical sensors\(^{38}\) are found in the literature.

In this way, herein are described new Tröger’s base scaffolds presenting different glycosyl moieties linked by the 1,2,3-triazole core through copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction. The corresponding 2,8-bis-alkyne \(3\) was isolated in 95% yield. In a second step, the copper-catalyzed azide–alkyne cycloaddition using azides derived from D-xylose, D-galactose, and D-mannose was performed.\(^{41}\) It was found that the efficiency of the CuAAC reaction was particularly dependent on the solvent probably due to solubility issues. Glycoconjugate \(4a\) was obtained by reaction of \(3\) with a D-xylose-derived azide, resulting in 66% yield when the reaction was performed in a 2:1 mixture of \(t\)-butanol/water as the solvent. For the D-galactose-derived azide, the best result was achieved in a binary mixture of dichloromethane/water, and the corresponding product \(4b\) was isolated in 61% yield. On the other hand, when the azide derived from D-mannose was used, the best result was obtained in dichloromethane/water to produce the derivative \(4c\) in 51% yield and the monofunctionalized derivative (16% yield), which were readily separated by column chromatography.\(^{40}\)

In order to improve the synthesis of \(4c\), it was decided to separately perform the deprotection reaction and the CuAAC cycloaddition. Thus, instead of adding TBAF to the reaction mixture in the CuAAC, we first removed both TMS groups from \(3\) using potassium carbonate in methanol/THF to afford the bis-terminal alkyne \(5\) in 92% yield. This compound was then subjected to the CuAAC reaction, and the desired glycoproduct \(4c\) was isolated in 75% yield (Scheme 3).\(^{40}\)

The photophysical investigation of the synthesized Tröger’s bases was performed by UV–vis absorption and steady-state and time-resolved fluorescence emission spectroscopies, respectively. The organic solvents with different dielectric constants (dichloromethane, 1,4-dioxane, ethyl acetate, ethanol, and acetonitrile) were used at a concentration of \(10^{-5}\) M. The absorption spectra of \(4a–c\) are presented in

**Scheme 1. Glycoconjugates Prepared in This Work from Tröger’s Base Scaffold**

**Scheme 2. Functionalization of the Tröger’s Base Scaffold**

\(^{(i)}\) PdCl\(_2\)(PPh\(_3\))\(_2\) (10 mol %), \(^{(ii)}\) Et\(_3\)N, trimethylsilylacetylene, 90 °C, 4 h, \(^{(iii)}\) CuSO\(_4\) (20 mol %), sodium ascorbate, \(^{(iv)}\) TBAF, \(t\)-BuOH/H\(_2\)O (2:1), rt, 48 h (for \(4a\)), TBAF, dichloromethane/H\(_2\)O (1:1), rt, 29 h (for \(4b\)) and TBAF, dichloromethane/H\(_2\)O (1:1), rt, 29 h (for \(4c\)).
The experimental data from UV−vis absorption spectroscopy are presented in Table 1.

The Troeger’s bases 4a−c present absorption maxima located around 275 nm with an almost absent solvatochromic effect, as already observed in the literature.42 In addition, the maxima location also indicates that the triazole moiety does not present effective conjugation with the aromatic rings present in the Troger’s bases, since it is found that values are very close to the Troger’s bases framework without any substituent.29 A particular behavior in ethanol was observed, where the absorption maxima blueshift is ∼10 nm due to specific solute-solvent interactions afforded by this polar protic media.

The UV−vis absorption results allowed obtaining ground-state parameters, such as the rate constant for emission ($k_{\text{e}}$), the oscillator strength ($f_e$), and the pure radiative lifetime ($\tau^0$) applying eqs 1−3.43,44 The respective values are summarized in Table 1.

$$f_e \approx 4.3 \times 10^{-9} \int \varepsilon \, d\tilde{v}$$

$$k_{\text{e}}^0 \approx 2.88 \times 10^{-9} \bar{\nu}_0^2 \int \varepsilon \, d\tilde{v}$$

$$\tau^0 = 1/k_{\text{e}}^0$$

In these equations, $\varepsilon$ (M$^{-1}$-cm$^{-1}$) is the extinction coefficient and $\int \varepsilon \, d\tilde{v}$ is the area under the absorption curve from a plot of $\varepsilon$ (M$^{-1}$-cm$^{-1}$) versus the wavenumber $\tilde{v}$ (cm$^{-1}$), which corresponds to a single electron oscillator. The rate constant ($k_{\text{e}}^0$) for emission can be related to $\varepsilon$ using eq 2, where the absorption maxima $\bar{\nu}_0$ is presented in cm$^{-1}$. In addition, the calculated pure radiative lifetime $\tau^0$ can be obtained from the rate constant from eq 3. The molar absorptivity coefficient $\overline{\varepsilon}$ values (10$^4$ L-mol$^{-1}$-cm$^{-1}$) and the calculated radiative rate constant ($k_{\text{e}}^0$) (10$^9$ s$^{-1}$) for all Troger’s bases indicate spin and symmetry-allowed electronic transitions, which could be related to $^1\pi \rightarrow \pi^*$. Moreover, the radiative lifetime show quite similar values ($\tau^0 \approx 1$ ns), suggesting that after excitation, these structures populate the same excited state, as already observed for different Troger’s bases.29

The normalized fluorescence emission spectra of Troger’s bases 4a−c are presented in Figure 3, obtained by exciting the compounds at the absorption maxima. The relevant data from this characterization are also presented in Table 1.

The glycoconjugates show a main fluorescence emission located around 350 nm with an almost absent solvatochromic effect, as already observed in the literature.42 In addition, the maxima location also indicates that the triazole moiety does not present effective conjugation with the aromatic rings present in the Troger’s bases, since it is found that values are very close to the Troger’s bases framework without any substituent.29 A particular behavior in ethanol was observed, where the absorption maxima blueshift is ∼10 nm due to specific solute-solvent interactions afforded by this polar protic media.

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The glycoconjugates show a main fluorescence emission located around 350 nm, indicating that the glycosyl moieties do not present any role on their excited-state photophysics. As already observed in the ground state, a small positive solvatochromic effect was observed from 1,4-dioxane to acetonitrile, showing that their dipole moments are larger in the excited state than in the ground state (i.e., $\mu_e \rightarrow \mu_h$). Concerning the fluorescence quantum yields, surprisingly
higher values could be obtained for the glycoconjugates 4a–c ($\Phi_{FL} \approx 0.2$) if compared to the values from the simpler Tröger’s base framework ($\Phi_{FL} \approx 0.06$). This result indicates that the glycosyl moieties are not efficient excited-state deactivation channels.

The excited-state dynamics of the Tröger’s bases was investigated by time-resolved fluorescence spectroscopy. The respective curves are depicted in Figure 4. The residuals are presented in the Supporting Information, and the relevant data are summarized in Table 2. Moreover, the radiative and nonradiative decay rate constants, $k_r$ and $k_{nr}$ respectively, were also estimated from eqs 4 and 5.

### Table 1. Photophysical Data of the UV–vis Spectra of the Tröger’s Bases 4a–c

| Tröger’s base | solvent | conc. | $\lambda_{abs}$ | $\epsilon$ | $f_e$ | $k^0_r$ | $r^0$ | $\lambda_{em}$ | $\Delta\lambda_{ST}$ | $\Phi_{FL}$ |
|---------------|---------|------|----------------|--------|------|--------|------|--------------|--------------|-----------|
| 4a            | 1,4-dioxane | 2.56 | 276            | 2.65   | 0.95 | 1.25   | 0.80 | 350          | 74/7660      | 0.21       |
|               | ethyl acetate | 5.03 | 275            | 1.51   | 0.91 | 1.20   | 0.84 | 346          | 71/7462      | 0.52       |
|               | dichloromethane | 3.14 | 276            | 2.38   | 0.90 | 1.19   | 0.84 | 358          | 82/8299      | 0.21       |
|               | ethanol      | 3.14 | 268            | 1.89   | 0.76 | 1.06   | 0.95 | 360          | 92/9536      | 0.36       |
|               | acetonitrile | 3.43 | 275            | 1.73   | 0.72 | 0.95   | 1.05 | 362          | 87/8739      | 0.16       |
| 4b            | 1,4-dioxane | 1.64 | 276            | 2.48   | 0.98 | 1.29   | 0.78 | 347          | 71/7413      | 0.12       |
|               | ethyl acetate | 2.10 | 276            | 2.63   | 0.93 | 1.22   | 0.82 | 346          | 70/7330      | 0.24       |
|               | dichloromethane | 3.47 | 276            | 2.83   | 0.92 | 1.21   | 0.83 | 352          | 76/7823      | 0.14       |
|               | ethanol      | 3.56 | 268            | 2.43   | 0.96 | 1.34   | 0.75 | 359          | 91/9458      | 0.22       |
|               | acetonitrile | 3.01 | 274            | 2.18   | 0.94 | 1.25   | 0.80 | 357          | 83/8485      | 0.16       |
| 4c            | 1,4-dioxane | 2.39 | 276            | 2.43   | 0.99 | 1.30   | 0.77 | 350          | 74/7660      | 0.16       |
|               | ethyl acetate | 2.34 | 273            | 3.45   | 0.92 | 1.23   | 0.81 | 348          | 75/7894      | 0.23       |
|               | dichloromethane | 4.37 | 276            | 2.07   | 0.89 | 1.16   | 0.86 | 355          | 79/8063      | 0.14       |
|               | ethanol      | 2.36 | 268            | 3.37   | 0.96 | 1.33   | 0.75 | 357          | 91/9583      | 0.19       |
|               | acetonitrile | 2.71 | 274            | 3.04   | 0.92 | 1.22   | 0.82 | 358          | 83/8431      | 0.16       |

The concentration is presented in $10^{-5}$ M; $\lambda_{abs}$ and $\lambda_{em}$ are the absorption and emission maxima, respectively (nm); $\epsilon$ is the molar absorptivity ($10^4$ M$^{-1}$·cm$^{-1}$); $f_e$ is the calculated oscillator strength; $k^0_r$ is the calculated radiative rate constant ($10^9$ s$^{-1}$); $\tau^0$ is the calculated pure radiative lifetime (ns); $\Delta\lambda_{ST}$ is the Stokes shift (nm/cm$^{-1}$); and $\Phi_{FL}$ is the relative fluorescence quantum yield.

### Figure 4. Fluorescence decay curves of Tröger’s bases 4a–c in (a) 1,4-dioxane and (b) acetonitrile (ca. $10^{-5}$ M). IRF = instrument response factor.

### Table 2. Time-Resolved Fluorescence Data of the Tröger’s Bases 4a–c

| Tröger’s base | solvent | $\tau$ | $A$ | $r^0$ | $k_r$ ($\times 10^7$) | $k_{nr}$ ($\times 10^8$) |
|---------------|---------|-------|-----|------|----------------------|------------------------|
| 4a            | 1,4-dioxane | 1.914 | 1.089 | 1.190 | 10.97                | 4.13                   |
|               | acetonitrile | 1.937 | 1.070 | 1.162 | 8.26                 | 4.34                   |
| 4b            | 1,4-dioxane | 1.431 | 1.251 | 1.137 | 8.39                 | 6.15                   |
|               | acetonitrile | 1.471 | 1.248 | 1.111 | 10.88                | 5.71                   |
| 4c            | 1,4-dioxane | 1.827 | 0.780 | 1.083 | 8.76                 | 4.60                   |
|               | acetonitrile | 1.954 | 1.056 | 1.178 | 8.19                 | 4.30                   |

$\tau$ is the experimental fluorescence lifetime (in ns), $A$ is the preexponential factor, $r^0$ indicates the quality of the exponential fit, $k_r$ ($\times 10^7$) is the calculated radiative decay constant (in s$^{-1}$), and $k_{nr}$ ($\times 10^8$) is the calculated nonradiative decay constant (in s$^{-1}$).

\[
radiative ~ decay ~ rate ~ constant: \quad k_r = \Phi_{FL}/\tau_f \tag{4}
\]

and

\[
nonradiative ~ decay ~ rate ~ constant: \quad k_{nr} = (1/\tau_f) - (\Phi_{FL}/\tau_f) \tag{5}
\]

where $\tau_f$ is the experimental fluorescence lifetime and $\Phi_{FL}$ is the fluorescence quantum yield. The fluorescence decay...
profiles indicated a monoexponential fluorescence lifetime with values between 1.4 and 2.0 ns with good χ² values. The solvent seems to not significantly affect the fluorescence lifetime of these compounds. In acetonitrile and 1,4-dioxane, it could be observed that the Tröger’s base 4c has a decrease in the fluorescence rate constant (kₐ) attributable to more effective nonradiative processes due to this moiety in the Tröger’s base scaffold. The nonradiative process showed to be higher for all compounds in the studied solvents, as expected.

BSA presents well-known photophysics characterized by an absorption located around 280 nm and a fluorescence emission around 340 nm related to the tryptophan residues. In this way, to investigate the interaction between the Tröger’s bases and BSA, its fluorescence quenching in the presence of 4a–c was studied. It is understood that this quenching can be associated to different methods, such as molecular rearrangements, energy transfer, excited-state reactions, ground-state complex formation, and collisional deactivation. In this way, the variation of fluorescence intensity of BSA in the presence of the compounds was measured using 280 nm as the excitation wavelength. Figure 5 depicts the suppression experiment using compound 4b as the BSA quencher. It is worth mentioning that the interaction studies with BSA were also performed for comparison with the Tröger’s base 1 in order to evaluate the glycosyl moiety role on the interaction between the Tröger’s bases and BSA.

It could be observed that as the concentration of the Tröger’s bases increases, the fluorescence intensity of BSA decreases, which already qualitatively indicates a significant interaction of these compounds with BSA. However, it can also be observed that the methylated Tröger’s base 1 (Figure 5a) does not present a linear relationship (R² = 0.941) over the entire range of studied concentration. On the other hand, compound 4b shows a linear relation between the fluorophore concentration and the BSA fluorescence intensity (R² = 0.989). In addition, the Tröger’s base 1 (Figure 5a) presented a lower reduction in the fluorescence intensity of BSA (14%) than the glycoconjugate 4b (67%). We would like to highlight that the additional glycoconjugates 4a and 4c also presented linear correlations with similar values in the fluorescence suppression of BSA (67 to 71%) (data not shown; see the Supporting Information), indicating that the carbohydrate moiety plays an important role in the interaction with BSA.

In order to better investigate the suppression mechanism between the Tröger’s bases and BSA, additional experiments were performed at different temperatures (25, 30, 35, and 40 °C) by applying the Stern–Volmer relation presented in eq 6:

\[
\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q]
\]

where F₀ and F are the fluorescence intensities of BSA in the absence and presence of certain amounts of the Tröger’s bases, respectively; Kₚ and Kₛᵥ are the bimolecular suppression and Stern–Volmer constants, respectively; and [Q] is the quencher concentration. In this equation, Kₛᵥ is related to the suppression efficiency, and τ₀ is the fluorophore lifetime in the absence of the suppressor, with values around 10⁻⁸ s to biomolecules. The relevant data are summarized in Table 3.

It can be observed that the constants Kₛᵥ and Kₚ independent on the temperature present values higher than 10¹¹ L·mol⁻¹·s⁻¹, which exceed the maximum value of 2.0 × 10¹⁰ L·mol⁻¹·s⁻¹ for the diffusion-controlled mechanism (dynamic quenching). Thus, it can be considered in this study that the suppression can be related to a static mechanism, where the formation of a glycoconjugate–BSA complex takes place in the ground state. Despite the observed mechanism for all studied compounds, the Tröger’s bases 1 presented lower values for the Kₛᵥ and Kₚ in comparison with its glycoconjugates 4a–c, which can be related to a weaker interaction with BSA.

It is worth mentioning that UV–vis absorption spectroscopy can also be a powerful tool to discuss the nature of the interaction between BSA and the Tröger’s base (static or dynamic). In this sense, since the dynamic mechanism involves only the excited state, it is expected that no changes will take place in the absorption spectrum (ground state). On the other hand, in the static mechanism, changes in the absorption spectrum are expected due to the formation of a new species (suppressor–BSA complex). Thus, equimolar amounts of the suppressors were added to a solution of BSA and evaluated by UV–vis absorption spectroscopy. In addition, in order to prove that the spectrum of BSA in the presence of the suppressor is not only the sum of the absorbances of BSA and the suppressor, the difference between the absorbance of the complex and the suppressor was also obtained (Figure 6).

It is observed that the BSA absorption bands in the presence and in the absence of the Tröger’s bases 4a–c differ from each other, which can be related to a change in the aromatic residues of BSA due to the formation of complexes BSA–
Table 3. Results from the Stern–Volmer Relation Form the Tröger’s Bases at Different Temperatures}\(^4\)

| Compound | T (°C) | \(F_0/F\) linear fit | \(R^2\) | \(K_{SV}\) | \(K_A\) |
|----------|--------|------------------------|--------|----------|--------|
| 1\(^{10}\) | 25     | \(F_0/F = 0.96 + 4.05 \times 10^3 Q\) | 0.988  | 4.05     | 4.05   |
|          | 30     | \(F_0/F = 0.98 + 4.03 \times 10^3 Q\) | 0.975  | 4.03     | 4.03   |
|          | 35     | \(F_0/F = 0.91 + 4.43 \times 10^3 Q\) | 0.983  | 4.43     | 4.43   |
|          | 40     | \(F_0/F = 0.94 + 3.72 \times 10^3 Q\) | 0.964  | 3.72     | 3.72   |
| 4a       | 25     | \(F_0/F = 0.60 + 5.25 \times 10^3 Q\) | 0.987  | 52.5     | 52.5   |
|          | 30     | \(F_0/F = 0.64 + 5.24 \times 10^3 Q\) | 0.980  | 52.4     | 52.4   |
|          | 35     | \(F_0/F = 0.60 + 5.05 \times 10^3 Q\) | 0.985  | 50.5     | 50.4   |
|          | 40     | \(F_0/F = 0.58 + 5.21 \times 10^3 Q\) | 0.985  | 52.1     | 52.1   |
| 4b       | 25     | \(F_0/F = 0.66 + 4.55 \times 10^3 Q\) | 0.991  | 45.5     | 45.5   |
|          | 30     | \(F_0/F = 0.67 + 4.46 \times 10^3 Q\) | 0.993  | 44.6     | 44.6   |
|          | 35     | \(F_0/F = 0.67 + 4.14 \times 10^3 Q\) | 0.989  | 41.4     | 41.4   |
|          | 40     | \(F_0/F = 0.60 + 4.35 \times 10^3 Q\) | 0.989  | 43.5     | 43.5   |
| 4c       | 25     | \(F_0/F = 0.84 + 3.50 \times 10^3 Q\) | 0.931  | 35.0     | 35.0   |
|          | 30     | \(F_0/F = 0.68 + 4.32 \times 10^3 Q\) | 0.933  | 43.2     | 43.2   |
|          | 35     | \(F_0/F = 0.72 + 3.63 \times 10^3 Q\) | 0.941  | 36.3     | 36.3   |
|          | 40     | \(F_0/F = 0.72 + 3.74 \times 10^3 Q\) | 0.940  | 37.4     | 37.4   |

\(K_A (\times 10^{11} \text{ L mol}^{-1} \text{ s}^{-1})\) is the bimolecular suppression constant, and \(K_{SV} (\times 10^3 \text{ L mol}^{-1})\) is the suppression constant. \(^{2,8}\)-Bis(methyl)-6H,12H-(5,11)-methanodibenzo[b,f][1,5]diazocine (Figure 1).

![Figure 6](image) UV–vis absorption spectra of BSA in the absence and presence of the Tröger’s bases (a) 1, (b) 4a, (c) 4b, and (d) 4c.

Tröger’s bases, agreeing with the assumption of the static mechanism. It is worth mentioning that this experiment also indicates that the Tröger’s base I, presented for comparison, shows close absorption spectra (intensity and maxima location), which can be related to a lower interaction with BSA, as already discussed in this study. Thus, considering the static mechanism, the binding constant \(K_A\) and the number of binding sites \(n\) between BSA and suppressor can be calculated from eq 7:\(^{31}\)

\[
\log \left(\frac{F_0 - F}{F}\right) = n \log Q + \log K_A
\]

Table 4 presents the results from the double logarithmic plot relating the fluorescence intensities from BSA and the quencher concentration. According to these results, it was observed that glycoconjugates 4a–c present a strong interaction with BSA due to the high values of binding constant \(K_A\) higher than \(10^5\) L mol\(^{-1}\). Further, the binding constants \(K_A\) for Tröger’s 4a and 4b bases increase with the temperature, and there are two binding sites with BSA. On the other hand, for glycoconjugate 4c, there is only one binding site with BSA, and there is no tendency for \(K_A\) with the temperature. The observed differences between glycoconjugates such as the magnitude of \(K_A\), number of binding sites, and linearity allow us to conclude that the glycosyl portion plays a fundamental role on the interaction with the protein. For the Tröger’s base containing the methyl group, this analysis was not conclusive, since the results of \(K_A\) and the number of binding sites present a great variation in a very random way.

In order to investigate the interactions between the Tröger’s base derivatives 4a–c and 1 with BSA, docking simulations were performed. It could be observed from the predicted binding energy \((\Delta G_{\text{bind}})\) summarized in Table 5 that glycoconjugates 4a, 4b, and 4c present a more spontaneous interaction with BSA than the Tröger’s base model 1. These results are correlated with the experimental data, which indicates that the glycoconjugates present the major reduction in the BSA fluorescence. In relation to the binding pose, the molecular docking show that 4a and 4b interact in the IB subdomain of BSA closely to Trp134 (∼10 Å) (Figure 7a,b), while compounds 4c and 1 binds in the II A region, interacting with Trp213 (3.4–6.4 Å) (Figure 7c,d). In general, the compounds demonstrated H-bonds, hydrophobic, and electrostatic interactions with the BSA protein. It is worth mentioning that the BSA domains I A (1–112), I B (113–195), II A (196–303), II B (304–383), III A (384–500), and III B (501–583) presented in Figure 7 were based on previous studies.\(^{52,53}\)

It could be observed that the H-bonds presented by the glycoconjugates 4a–c are essential for the better binding affinity to BSA. Likewise, the Tröger’s base 1 that does not show H-bonds presented low BSA fluorescence quenching.

In order to investigate the interactions between the Tröger’s base derivatives 4a–c and 1 with BSA, docking simulations were performed. It could be observed from the predicted binding energy \((\Delta G_{\text{bind}})\) summarized in Table 5 that glycoconjugates 4a, 4b, and 4c present a more spontaneous interaction with BSA than the Tröger’s base model 1. These results are correlated with the experimental data, which indicates that the glycoconjugates present the major reduction in the BSA fluorescence. In relation to the binding pose, the molecular docking show that 4a and 4b interact in the IB subdomain of BSA closely to Trp134 (∼10 Å) (Figure 7a,b), while compounds 4c and 1 binds in the II A region, interacting with Trp213 (3.4–6.4 Å) (Figure 7c,d). In general, the compounds demonstrated H-bonds, hydrophobic, and electrostatic interactions with the BSA protein. It is worth mentioning that the BSA domains I A (1–112), I B (113–195), II A (196–303), II B (304–383), III A (384–500), and III B (501–583) presented in Figure 7 were based on previous studies.\(^{52,53}\)

It could be observed that the H-bonds presented by the glycoconjugates 4a–c are essential for the better binding affinity to BSA. Likewise, the Tröger’s base 1 that does not show H-bonds presented low BSA fluorescence quenching. In
interaction of the Troger's base with the protein BSA. It was described, in this investigation, the modification of the Troger's base scaffold with carbohydrates. Both moieties have been linked together efficiently by a 1,2,3-triazole through copper-catalyzed azide–alkyne cycloaddition of a Troger's base-alkyne and carbohydrate-derived azides. The Troger's bases presented absorption maxima located in the UV region with an almost absent solvatochromic effect. The Troger's bases presented a main emission located around 350 nm. The unexpected higher fluorescence quantum yields if compared to the values from the Troger's base framework indicated that the glycosyl moieties are not efficient excitation-state deactivation channels. The time-resolved fluorescence spectroscopy showed a monoexponential time decay with lifetime values between 1.4 and 2.0 ns with good $\chi^2$ values. BSA fluorescence suppression was observed in glycoconjugates 4a–c mainly due to the carbohydrate moiety, which presented a higher Stern–Volmer constant despite the simpler Troger's base 1.

### EXPERIMENTAL SECTION

**General Information.** Air-sensitive reactions were carried out in oven-dried glassware equipped with tightly fitted rubber septa. In these reactions, a positive pressure of dry argon was applied. Thin-layer chromatography (TLC) was performed using Silica Gel 60 F254. Column chromatography was performed using Silica Gel 60 Å (70–230 mesh). Carbohydrate-derived azides were synthesized according to the literature. NMR spectra were recorded in CDCl$_3$ solution on a Varian VNMRS 300 or 400 MHz or a Bruker 400 MHz spectrometer. Assignment of chemical shifts is based on standard NMR experiments and reported in parts per million (ppm) related to tetramethylsilane ($\delta = 0.00$ ppm in $^1$H NMR) or from the solvent peak of CDCl$_3$ ($\delta = 77.23$ ppm in $^{13}$C NMR). The data are presented as follows: chemical shift ($\delta$), multiplicity, coupling constant ($J$) in Hz, and the integrated intensity. $^{13}$C NMR spectra were recorded at 75 or 100 MHz in CDCl$_3$ solution. The multiplicities are given as s (singlet), d (doublet), t (triplet), dd (double doublet), m (multiplet), q (quartet), and br (broad singlet). High-resolution mass spectrometry with electrospray ionization (HRMS-ESI).

### CONCLUSIONS

It was described, in this investigation, the modification of the Troger's base scaffold with carbohydrates. Both moieties have been linked together efficiently by a 1,2,3-triazole through copper-catalyzed azide–alkyne cycloaddition of a Troger's base-alkyne and carbohydrate-derived azides. The Troger's bases presented absorption maxima located in the UV region with an almost absent solvatochromic effect. The Troger's bases presented a main emission located around 350 nm. The unexpected higher fluorescence quantum yields if compared to the values from the Troger's base framework indicated that the glycosyl moieties are not efficient excitation-state deactivation channels. The time-resolved fluorescence spectroscopy showed a monoexponential time decay with lifetime values between 1.4 and 2.0 ns with good $\chi^2$ values. BSA fluorescence suppression was observed in glycoconjugates 4a–c mainly due to the carbohydrate moiety, which presented a higher Stern–Volmer constant despite the simpler Troger's base 1.

### Table 4. Results from Eq 6 from Troger's Bases 1 and 4a–c

| compound | $T$ (°C) | $\log(K_0 - F/F) = 10.12 + 2.24 \log[Q]$ | $K_a$ (L·mol$^{-1}$) | $n$ |
|----------|---------|--------------------------------------|------------------|-----|
| 1        | 25      | 1.41 × 10$^{10}$                     | 2.24             |     |
|          | 30      | 1.62 × 10$^{4}$                      | 1.16             |     |
|          | 35      | 3.95 × 10$^1$                        | 0.59             |     |
|          | 40      | 8.37 × 10$^{-2}$                     | 2.01             |     |
| 4a       | 25      | 2.02 × 10$^{-2}$                     | 1.61             |     |
|          | 30      | 4.45 × 10$^{-2}$                     | 1.68             |     |
|          | 35      | 8.36 × 10$^{-2}$                     | 1.75             |     |
|          | 40      | 1.30 × 10$^{-10}$                    | 2.24             |     |
| 4b       | 25      | 2.52 × 10$^{-1}$                     | 1.64             |     |
|          | 30      | 2.27 × 10$^{-1}$                     | 1.86             |     |
|          | 35      | 9.58 × 10$^{-1}$                     | 2.00             |     |
|          | 40      | 5.64 × 10$^{-1}$                     | 2.18             |     |
| 4c       | 25      | 2.20 × 10$^{-1}$                     | 1.19             |     |
|          | 30      | 5.55 × 10$^{-1}$                     | 1.28             |     |
|          | 35      | 4.36 × 10$^{-1}$                     | 1.28             |     |
|          | 40      | 3.11 × 10$^{2}$                      | 1.25             |     |

”$K_a$ (L·mol$^{-1}$)” is the binding constant, and $n$ is the binding sites.

### Table 5. Amino Acids Residues Involved in the Interactions between BSA and the Troger's Base Derivatives 1 and 4a–c and the Predicted Binding Energy Obtained from the Docking

| Troger's base | hydrophobic | electrostatic | H-bonds | $\Delta G_{\text{bind}}$ (kcal·mol$^{-1}$) | $K_a$ $^\dagger$ |
|---------------|-------------|---------------|---------|------------------------------------------|-----------------|
| 1             | Arg198 and Arg256 | Lys136, Lys114, and Arg185 | Arg144 | -8.1 | 1.16 × 10$^5$ |
| 4a            | Arg185      | Arg194, Arg217, and Asp450 | Arg198 and Ser442 | -11.8 | 2.24 × 10$^7$ |
| 4b            | Arg115, Pro117, Leu122, and His145 | Lys16, Lys114, and Arg185 | Arg198 and Ser442 | -13.3 | 1.78 × 10$^{-10}$ |
| 4c            | Tyr149, Lys187, and Pro440 | Lys136, Lys114, and Arg185 | Arg198 and Ser442 | -11.6 | 3.14 × 10$^{-7}$ |

“$K_a$ refers to the dissociation constant between BSA and ligands obtained from $K_a = \exp((\Delta G \times 1000)/(R \times T))$, where $R$ is 1.98719 cal and $T$ is 298.15 K.
performing using a constant BSA concentration (11 μM in phosphate buffer solution, pH 7.2). In this study, different amounts of the dye solutions (5–50 μM in PBS) were added. The fluorescence emission spectra were obtained at 25 °C and under an excitation wavelength located at 280 nm. For the experiments at different temperatures, the samples were kept in a water bath, with a thermometer-controlled temperature of one decimal place.

**Synthesis.** 2,8-Diodo-6H,12H-5,11-methanodibenzo-[b,fl][1,5]diazocine (2). In an open round-bottom flask containing 10 mL of TFA at −15 °C, p-iodoaniline (3 mmol) and p-formaldehyde (4.5 mmol) were added. After the addition, the mixture was stirred for 24 h at room temperature. After this time, the reaction mixture was poured into cold water, and ammonium hydroxide was added until pH = 8 was reached. The crude product was filtered, dried, and purified by column chromatography using dichloromethane as the eluent, and the pure product was obtained as a yellow solid in 37% yield (0.260 g). mp 177–180 °C. 1H NMR (300 MHz, CDCl3) δ (ppm): 7.45 (dd, J = 8.5, 2.0 Hz, 2H), 7.23 (d, J = 2.0 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 4.61 (d, J = 16.7 Hz, 2H), 4.23 (s, 2H), 4.08 (d, J = 16.7 Hz, 2H). 13C NMR (75.5 MHz, CDCl3) δ (ppm): 147.7, 136.6, 135.9, 130.3, 127.2, 87.8, 66.7, 58.3.

2,8-Bis[(trimethylsilyl)ethenyl]-6H,12H-5,11-methanodibenzo-[b,fl][1,5]diazocine (3). In a dry tube, iodinated Tröger’s base 1 (1 mmol), CuI (8 mol %), PPh3 (9 mol %), PdCl2[PPh3]2 (10 mol %), EtN (4 mL), and ethynyltrimethylsilane (4 mmol) were added under an N2 atmosphere. The tube was sealed and stirred at 90 °C for 4 h. Finally, the reaction was cooled at room temperature, ethyl acetate was added, and the mixture was filtered under silica. The solvent was removed, and product 2 was obtained as a yellow solid in 95% yield (0.475 g). mp 194–196 °C. 1H NMR (300 MHz, CDCl3) δ (ppm): 7.24 (dd, J = 8.2, 2.0 Hz, 2H), 7.03 (d, J = 2.0 Hz, 2H), 7.02 (d, J = 8.2 Hz, 2H), 4.61 (d, J = 16.7 Hz, 2H), 4.26 (s, 2H), 4.10 (d, J = 16.7 Hz, 2H), 0.21 (s, 18H). 13C NMR (75.5 MHz, CDCl3) δ (ppm): 148.3, 131.0, 130.8, 127.6, 124.8, 118.6, 104.7, 93.4, 66.8, 58.5.

**General Procedure for the CuAAC.** In an open flask were added the Tröger’s base 3 (1.0 equiv), the appropriate carbohydrate-derived azide (2.0 equiv), CuSO4·5H2O (0.2 equiv), sodium ascorbate (0.4 equiv), and the appropriate solvent (as indicated in Scheme 1). After stirring for 5 min, TBAF (3 equiv, 1 M solution in THF) was added, and the reaction mixture was stirred for the time indicated in Scheme 1. The crude product was extracted with dichloromethane. The organic layer washed with 0.1 M EDTA solution, dried with Na2SO4 and filtered, and the solvent was removed under vacuum. The crude product was purified by column chromatography eluting with a gradient of dichloromethane and acetone.

**Glycoconjugate 4a.** Pale yellow solid, yield: 0.100 g, 66%. mp 152–155 °C. FTIR (KBr, cm−1): 3434, 3132, 2987, 2937, 2898, 1492, 1217, 1072. 1H NMR (300 MHz, DMSO-d6) δ (ppm): 8.42 (s, 2H), 7.62 (dd, J = 8.2 Hz and 1.5 Hz, 2H), 7.46 (d, J = 1.5 Hz, 2H), 7.21 (d, J = 8.2 Hz, 2H), 5.88 (d, J = 3.8 Hz, 2H), 5.67 (d, J = 5.0 Hz, 2H), 4.71 (d, J = 17.0 Hz, 2H), 4.47 (d, J = 3.8 Hz, 2H), 4.45–4.38 (m, 4H), 4.30 (s, 2H), 4.23 (d, J = 17.0 Hz, 2H), 4.08 (dd, J = 5.0 Hz and 2.6 Hz, 4H), 1.33 (s, 6H), 1.22 (s, 6H). 13C NMR (75.5 MHz, DMSO-d6) δ (ppm): 147.8, 146.2, 146.1, 128.5, 125.9, 125.3, 124.1, 123.6, 123.5, 121.4, 110.9, 104.5, 85.0, 79.1, 73.5, 66.2.
Glycoconjugate 4b. Pale yellow solid, yield: 0.180 g, 61%. mp 275–280 °C. FTIR (KBr, cm⁻¹): 3143, 2987, 2937, 2906, 1383, 1213, 1066. H NMR (400 MHz, CDCl₃) δ (ppm): 7.83 (s, 1H), 7.82 (s, 1H), 7.56 (d, J = 8.2 Hz, 2H), 7.43 (s, 2H), 7.17 (d, J = 8.2 Hz, 2H), 5.49 (d, J = 1.6 Hz, 2H), 4.73 (d, J = 16.8 Hz, 2H), 4.65–4.58 (m, 4H), 4.42 (dd, J = 1.7 Hz and 8.2 Hz, 2H), 4.34 (s, 2H), 3.43 (dd, J = 0.5 Hz and 2.4 Hz, 2H), 2.42 (d, J = 16.8 Hz, 2H), 4.17 (d, J = 7.8 Hz, 4H), 1.47 (s, 6H), 1.34 (s, 6H), 1.25 (s, 6H). 13C NMR (100.5 MHz, CDCl₃) δ (ppm): 174.8, 174.1, 128.2, 128.2, 126.8, 126.7, 125.3, 120.4, 109.8, 109.0, 96.2, 71.2, 70.7, 70.3, 67.0, 58.7, 50.5, 26.0, 25.9, 24.8. HRMS (ESI-MS) calc’d for C₄₃H₅₃N₈O₁₀ [M + H]+ 841.3885, found 841.3820.

Glycoconjugate 4c. Pale yellow solid, yield: 51%. mp 204–208 °C. FTIR (KBr, cm⁻¹): 3168, 2985, 2941, 2898, 1375, 1209, 1070. H NMR (400 MHz, CDCl₃) δ (ppm): 7.95 (s, 1H), 7.95 (s, 1H), 7.58 (dd, J = 8.8 Hz and 1.6 Hz, 2H), 7.55 (dd, J = 8.8 Hz and 1.6 Hz, 2H), 7.42 (d, J = 1.6 Hz, 1H), 7.40 (d, J = 1.6 Hz, 1H), 7.18 (d, J = 8.2 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 6.06 (d, J = 3.5 Hz, 2H), 4.91 (dd, J = 5.8 Hz and 3.5 Hz, 2H), 4.84 (dd, J = 5.2 Hz and 3.5 Hz, 2H), 4.73 (d, J = 16.8 Hz, 2H), 4.47 (ddd, J = 7.6 Hz, 6.6 Hz and 4.7 Hz, 2H), 4.34 (s, 2H), 4.24 (d, J = 16.8 Hz, 2H), 4.09 (dd, J = 8.9 Hz and 6.3 Hz, 2H), 4.03 (dd, J = 8.9 Hz and 4.3 Hz, 2H), 3.75 (dd, J = 7.6 Hz and 3.5 Hz, 2H), 1.54 (s, 3H), 1.52 (s, 3H), 1.42 (s, 6H), 1.36 (s, 6H), 1.29 (s, 3H), 1.28 (s, 3H). 13C NMR (100.5 MHz, CDCl₃) δ (ppm): 148.1, 147.9, 128.2, 126.4, 126.3, 125.4, 125.3, 125.0, 124.3, 119.9, 113.7, 113.6, 109.5, 88.8, 79.6, 79.2, 72.6, 66.9, 66.7, 58.8, 58.7. 26.9, 25.6, 25.5, 25.1, 24.0. HRMS (ESI-MS) calc’d for C₄₃H₅₃N₈O₁₀ [M + H]+ 841.3885, found 841.3820.

Docking. The structure of bovine serum albumin (BSA) was obtained from the Protein Data Bank.54,66 The addition of chain B, water, and other small molecules, as well as hydrogen atoms was performed by the Chimera 1.8 software.68 The software AvoGadro 1.1.1 was used to build the chemical structure of the Tröger’s base derivatives57 following the semiempirical PM6 geometry optimization (Program MOPAC2012).67 Here, as a model of interaction, only the R isomers were tested. The Tröger’s bases and BSA were generated in the pdbqt format by AutoDockTools. In this methodology, the Tröger’s bases were considered flexible (with PM6 charges), and BSA was considered rigid (with Gasteiger charges).70 The blind docking was performed by the AutoDock Vina 1.1.1 software71 with a gridbox of 96 × 64 × 88 and the coordinates x = 9.46, y = 23.36, and z = 98.15 (exhaustiveness of 150). For the binding pose, the respective conformation with the lowest binding free energy (ΔG) was selected. The results from docking were analyzed using the Discovery Studio Visualizer 17.2.0 (DSV) software.72

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01857.

Original spectra from spectroscopic characterization and additional photophysical data (PDF)
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