Synthesis of fluorine-containing tetraketones and diketo esters and luminescence-spectral properties of their complexes with lanthanide ions

D. V. Romanov,^{a*} N. V. Vasil'ev,^a A. I. Lyamin,^b N. P. Ivanovskaya,^b and N. S. Osin^b

^aMilitary University of Radioactive, Chemical and Biological Defense, 13 Brigadirsky per., 105005 Moscow, Russian Federation. Tel.: +7 (495) 265 9318

^bState Scientific Center of the Russian Federation "State Research Institute of Biological Engineering," building 1, 75 Volokolamskoe shosse, 125424 Moscow, Russian Federation. Tel.: +7 (495) 490 5704. E-mail: immunosc@online.ru

The Claisen condensation of diesters of fluorinated dicarboxylic acids with 2-acetylnaphthalene affords the corresponding naphthyl-containing tetraketones and diketo esters. The luminescence-spectral properties and stability constants of complexes of these compounds with Eu^{3+} ions were estimated. The characteristics of the complexes are comparable with those of fluorinated β -diketones containing chromophoric substituents, which are widely used in luminescence analysis. The synthesized naphthyl-containing diketo esters are efficiently conjugated with proteins and can be used for detection of biospecific interactions.

Key words: β -diketones, tetraketones, diketo esters, lanthanides, fluorine-containing compounds, luminescence analysis.

Metal complexes with fluorine-containing β -diketones as ligands find wide use in various analytical applications.¹⁻³ In particular, similar rare-earth metal (REM) complexes are used in variants for luminescence detection of biospecific interactions.^{3–8}

As other lanthanide chelates they are characterized by unique luminescence-spectral properties (considerable Stokes shift >200 nm, narrow luminescence emission band ~14 nm, long lifetime of the excited state τ up to 800 µs). At the same time, these compounds have additional advantages: high quantum efficiency ($\epsilon \phi$) and longer-wavelength position of the luminescence excitation maximum. All these data are very significant for sensitivity increasing and specificity of analysis and for possibility of its instrumental realization.

At the same time, β -diketonate complexes of REM are not sufficiently stable for non-separable analysis or analysis *in situ*. The binding constant of the europium ion (Eu³⁺) by the most known complexone of this type, namely, naphthoyltrifluoroacetone (NTA),³ is only ~10⁷ L mol⁻¹. Therefore, this complexone is used only in high excess over Eu³⁺ ions in the variant of dissociation enhanced lanthanide fluorescence immunoassay (DELFIA). The introduction of additional β -diketonate residues (two^{5,6,8} or even four⁷) into a chelating molecule for enhancing the dentate character of ligands was used in recent attempts to increase the stability of complexes. However, the complexones recommended for use in nondissociation assay are not efficient enough because they require a high excess of Eu^{3+} and are multiply inferior to NTA in quantum efficiency.

Thus, the search for complexones with high binding constants and quantum efficiency of luminescence remains topical. Therefore, it seemed of interest to prepare models of bis- β -diketonate complexones with different lengths of the fluorine-containing chain and estimate their luminescence-spectral and complexing properties.

Results and Discussion

Naphthyl-containing tetraketones 1a-d, whose β -diketonate moieties are separated by a perfluoroalkylene chain $(CF_2)_n$ of different length (n = 1, 2, 4, 6) were synthesized according to the Claisen method (Scheme 1). The reactions of dimethyl perfluorodicarboxylates with 2-acetylnaphthalene were carried out in the presence of anhydrous sodium alkoxide in ether. The maximum conversion of the reactants was achieved on refluxing and stirring of the reaction mixture for 3–4 h. In all cases, diketo esters 2a-d are formed along with the expected tetraketones. Unlike compounds 2a-d, tetraketones 1a-dare virtually insoluble in ether and other solvents with low polarity, and this property was used to isolate them. Diketo esters 2a-d were additionally purified by fractionation

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in vacuo. The ¹H and ¹⁹F NMR spectra show that tetraketones 1a-d and diketo esters 2a-d exist exclusively in the enole form.



n = 1 (**a**), 2 (**b**), 4 (**c**), 6 (**d**)

It should be mentioned that the formation of diketo esters of similar structure has not previously^{5,9} been reported in the synthesis of different tetraketones.

The chelating properties of the synthesized compounds toward lanthanide ions were estimated by luminescencespectral methods. The long-wavelength absorption band values and molar absorption coefficients of the complexes of tetraketones 1a-d and diketo esters 2a-d with Eu³⁺ are presented in Table 1. An ion : ligand ratio of 20 : 1, at which the formation of the 1 : 1 complex is most probable, was used in the estimation of the luminescencespectral characteristics. The formation of similar complexes is also most probable for ligand conjugation with a biological object labeled by this ligand.

As follows from the data presented, the spectral characteristics of the Eu³⁺ complexes with compounds **1a–d** and **2a–d** are close to those for the Eu³⁺ complex with NTA: their long-wavelength absorption maximum lies at 340–350 nm. The molar absorption coefficients for diketo esters **2a–d** are close to the value for NTA. The ε value for tetraketones **1a–d** is 1.5–2 times higher, and a tendency for increasing the molar absorption coefficients with an increase in the number of difluoromethylene groups is observed.

When europium salt are added to aqueous solutions of trioctylphosphine oxide (TOPO), which is a synergetic

Table 1. Long-wavelength absorption band values (λ_{max}) and
molar absorption coefficients (ϵ) for the Eu ³⁺ complexes of the
studied compounds in aqueous media ^a

Comp	-	I ^b	IIc		
lex- one	λ _{max} /nm	$\epsilon \cdot 10^{-4}$ /L mol ⁻¹ cm ⁻¹	λ _{max} /nm	$\epsilon \cdot 10^{-4}$ /L mol ⁻¹ cm ⁻¹	
NTA	_	_	334→336	2.00→2.10	
1a	346→348	2.55→2.62	346→350	3.00→3.05	
1b	340	1.03→1.10	340→344	2.85→3.10	
1c	340	2.40	340→342	3.80	
1d	342	2.80	340→342	3.90	
2a	335→340	1.30→1.25	338→338	2.00→2.20	
2b	_	_	334→340	2.00→1.92	
2c	340	1.15	336→340	1.78→1.92	
2d	342	1.70	338→340	2.45→2.52	

^{*a*} The arrow means a change in λ_{max} and ε upon the addition of EuCl₃ (final concentration $2 \cdot 10^{-4}$ mol L⁻¹) to the complexone (10^{-5} mol L⁻¹).

^b Distilled water.

 c Solution of trioctylphosphine oxide (5 \cdot 10 $^{-5}$ mol L $^{-1}$) and Triton X-100 (0.1%) in water.

agent favoring Eu³⁺ insulation in a complex from the quenching effect of water molecules, and micelle-forming agent Triton X-100,³ the compounds of the studied series in a wide interval of stoichiometric ratios exhibit emission at 613–616 nm (characteristic of Eu³⁺) for excitation of the complexone in the absorption region (~340 nm) beginning from concentrations about 10^{-10} mol L⁻¹.

The luminescence intensities and lifetimes of the complexes formed at the stoichiometric ratio ligand : ion = 1 : 500 (10^{-8} mol L⁻¹ : 5 · 10^{-6} mol L⁻¹) are given in Table 2.

As follows from the data obtained, no explicit dependence is observed for the lifetime and luminescence intensity of the Eu³⁺ complexes of the synthesized compounds on the difluoromethylene chain length and the number of C=O groups in the β -diketonate fragments. The highest luminescence intensity (154 rel. units) is achieved for ligand **1d** (six CF₂ groups and four C=O groups), while the τ parameter for this ligand is one of the lowest (666 µs). Ligand **2a** (one CF₂ group and two C=O groups) forms a complex with the lowest luminescence intensity (44 rel. units) but higher τ value (724 µs). The highest τ value (856 µs) belongs to the complex based on ligand **1b** (two CF₂ groups and four C=O groups); however, its luminescence intensity is insignificant (63 rel. units).

The binding constants for the Eu^{3+} ion and complexones (Table 3) were determined from the luminescence characteristics of the complexes. The data obtained show that the increase in the dentate character of the complexones, which is achieved in molecules of tetra-

Table 2. Parameters of prolong luminescence of the Eu³⁺ complexes of the synthesized compounds (fluorescence relative to NTA (F_{rel}) and luminescence lifetime (τ))*

Complexone	п	Z***	$F_{\rm rel}$ (%)	τ/μs
NTA	1	2	100±56	827±37
			(6)	[0.9999]
1a	1	4	81±9	644±30
			(3)	[0.9999]
1b	2	4	63±35	856±9
			(6)	[0.9998]
1c	4	4	79±14	615±22
			(4)	[0.9990]
1d	6	4	154±18	666±1
			(2)	[0.9999]
2a	1	2	44±36	724±16
			(6)	[0.9998]
2b	2	2	70 ± 25	724 ± 40
			(6)	[0.9998]
2c	4	2	56±31	760 ± 5
			(3)	[0.9999]
2d	6	2	70 ± 4	685±2
			(2)	[0.9999]

* The number of independent entries performed on different days is given in parentheses, and the pair correlation coefficient calculated using the least-squares method by approximation of the obtained data according to the monoexponential law of luminescence intensity decay is presented in brackets.

** Here and in Table 3, the number of the C=O groups in the β -diketonate moieties.

ketones 1a-d, affects dramatically the binding constants: they are increased by four and more orders of magnitude. Some tendency for increasing the binding constant with an elongation of the difluoromethylene chain is also ob-

Table 3. Binding constants of the synthesized luminescent complexones with the Eu^{3+} ion

Complexone n z Binding constant /L mol ⁻¹ NTA — 2 $(1.7-1.9) \cdot 10^7$ 1a 1 4 $\geq 10^{11}$ 1b 2 4 $\geq 10^{11}$ 1c 4 4 $\geq 10^{11}$ 1d 6 4 $\geq 10^{11}$ 2a 1 2 $(0.5-3.0) \cdot 10^7$ 2b 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$				
NTA - 2 $(1.7-1.9) \cdot 10^7$ 1a 1 4 $\ge 10^{11}$ 1b 2 4 $\ge 10^{11}$ 1c 4 4 $\ge 10^{11}$ 1d 6 4 $\ge 10^{11}$ 2a 1 2 $(0.5-3.0) \cdot 10^7$ 2b 2 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$	Complexone	п	z	Binding constant /L mol ⁻¹
1a 1 4 $\geq 10^{11}$ 1b 2 4 $\geq 10^{11}$ 1c 4 4 $\geq 10^{11}$ 1d 6 4 $\geq 10^{11}$ 2a 1 2 $(0.5-3.0) \cdot 10^7$ 2b 2 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$	NTA	_	2	$(1.7 - 1.9) \cdot 10^7$
1b 2 4 $\geq 10^{11}$ 1c 4 4 $\geq 10^{11}$ 1d 6 4 $\geq 10^{11}$ 2a 1 2 $(0.5-3.0) \cdot 10^7$ 2b 2 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$	1a	1	4	$\geq 10^{11}$
1c 4 4 $\geq 10^{11}$ 1d 6 4 $\geq 10^{11}$ 2a 1 2 $(0.5-3.0) \cdot 10^7$ 2b 2 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$	1b	2	4	$\geq 10^{11}$
1d 6 4 $\geq 10^{11}$ 2a 1 2 $(0.5-3.0) \cdot 10^7$ 2b 2 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$	1c	4	4	$\geq 10^{11}$
2a 1 2 $(0.5-3.0) \cdot 10^7$ 2b 2 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$	1d	6	4	$\geq 10^{11}$
2b 2 2 $(0.5-2.0) \cdot 10^7$ 2c 4 2 $(2.0-3.3) \cdot 10^7$ 2d 6 2 $(3.6-10.0) \cdot 10^7$	2a	1	2	$(0.5-3.0) \cdot 10^7$
2c 4 2 $(2.0-3.3)\cdot 10^7$ 2d 6 2 $(3.6-10.0)\cdot 10^7$	2b	2	2	$(0.5-2.0) \cdot 10^7$
2d 6 2 $(3.6-10.0) \cdot 10^7$	2c	4	2	$(2.0-3.3) \cdot 10^7$
20 5 2 (5.6 16.6) 10	2d	6	2	$(3.6-10.0) \cdot 10^7$

Note. The constants were determined in aqueous media with TOPO, Triton X-100, and Tris buffer (pH 7.2) from the dependence of the luminescence intensity of the system on the concentration at the ratios ligand : ion = 1 : 1 (for 1a-d) and ligand : ion = 1 : (100-1000) and 1 : 1 (for 2a-d).

served for diketo esters 2a-d. Compound 2d (six CF₂ groups) forms complexes that are threefold more stable than those with NTA. The elongation of the difluoromethylene chain sterically facilitates, most likely, a more favorable arrangement of the groups (most probably, the diketonate moieties) involved in complex formation.

Note that none of the synthesized compounds forms luminescent complexes with the Tb^{3+} ion.

Diketo ester **2d** was examined as a potential marker of biological structures. In its 20-fold mole excess in a conjugating medium relatively to the labeled molecule of bovine serum albumin (BSA), conjugates containing about five molecules of marker **2d** per one protein molecule were obtained. The conjugate yield based on protein was 71%. Conjugation occurs, most likely, due to acylation of the amino groups of the protein by the highly reactive methoxycarbonyl groups of compound **2d**.

The ability of the complexone to form luminescent complexes with Eu^{3+} in the BSA-bonded state was estimated in 0.05 *M* solutions of Tris buffer (pH 7.4) and after TOPO (5 \cdot 10⁻⁵ mol L⁻¹) and Triton X-100 (0.1%) were added to this solution. The results are given in Table 4.

As follows from the data obtained, in the absence of the additional insulating shell, which is optimally created by TOPO with Triton X-100, the luminescence of the complex is quenched with water. However, the presence of BSA and, the more so, conjugation with it favor insulation, which is especially pronounced at high concentrations of the complex (for the luminescence intensities in parentheses of the second column, see Table 4).

In all cases, the addition of TOPO increases the luminescence of Eu^{3+} in the complex with ligand **2d**, being the lowest in the conjugated state. Evidently, the sites of ligand binding in the conjugated and BSA-adsorbed states differ, which appears as an insufficient increase in the luminescence in the presence of TOPO.

Table 4. Luminescence intensity (1) of the $2d \cdot Eu^{3+}$ complex in the free and BSA-bonded states in different media*

Complex	I (rel. units)				
2d • Eu ³⁺	TB**	TB + + TOPO	TB + TOPO + + Triton X-100		
Free	7 (70)	1070 (10500)	1350		
In the presence of BSA	7 (170)	820 (14900)	1090		
In the conjugate with BSA	7 (365)	250 (6550)	80		

* [2d] = 10^{-8} (3.2· 10^{-7}) mol L⁻¹, [Eu³⁺] = $5 \cdot 10^{-6}$ mol L⁻¹, [BSA] = $2 \cdot 10^{-9}$ (6.3· 10^{-8}) mol L⁻¹ (the *I* values in parentheses correspond to the concentrations given in parentheses); λ_{exc} = 340 nm, λ_{em} = 614 nm, t_d = 0.05 (0.1) ms, t_g = 1 ms (see Experimental).

** Tris buffer.

The addition of Triton X-100 to the free complexone results in the further increase in the luminescence intensity (in the presence of BSA, this is less pronounced). However, an additional (almost threefold) quenching of luminescence is observed in the conjugated state. In this case, the Eu^{3+} ions with TOPO are redistributed, most likely, from the conjugated complexone to Triton X-100 micelles containing no ligands, due to which the luminescence intensity decreases additionally.

The luminescence lifetimes of the complex of **2d** with Eu^{3+} in the free, adsorbed, and BSA-conjugated states in the presence of TOPO are almost the same, being 390, 394, and 400 µs, respectively, which agrees with the

 τ values obtained for the complexones of this series in the absence of Triton X-100.

The estimations for the newly synthesized diketo esters **2a**—**d** show that they have comparable characteristics with fluorinated β -diketones containing the chromophore substituents, which are widely used for luminescence analysis. At the same time, these compounds possess a basically novel property: they are efficiently conjugated with protein. The complexing properties of tetraketones **1a**—**d** undergo jumpwise changes due to an increase in the dentate character of these molecules, which can find use in the practice of fluorescence immunoassay.

Table 5. Physicochemical characteristics, yields, elemental analysis data, and ¹H, ¹⁹F NMR and IR spectra of the synthesized compounds

Com-	B.p.	M.p.	Yield	Found	(%)	Molecular	NMR, δ (J/Hz)		IR,
po-	/°C	/°C		Calcula	ted	formula	1H	¹⁹ F	v/cm^{-1}
und	(p/Torr)		(%)	С	Н				
1a	_	171	12	<u>72.95</u> 72.97	<u>4.31</u> 4.05	$C_{27}H_{18}F_2O_4$	6.9 (s, 1 H, =CH); 7.6 (m, 2 H, H arom.); 7.9 (m, 4 H, H arom.); 8.6 (s, 1 H, H arom.)	39.8 (s, 2 F, CF ₂)	1581 (C=O)
1b	_	180—190 (decomp.)	28	<u>67.75</u> 68.02	<u>3.27</u> 3.64	$C_{28}H_{18}F_4O_4$	6.8 (s, 1 H, =CH); 7.6 (m, 2 H, H arom.); 7.9 (m, 4 H, H arom.); 8.5 (s, 1 H, H arom.)	42.0 (s, 4 F, 2 CF ₂)	1610 (C=O)
1c	_	150—154	11	<u>60.28</u> 60.60	<u>3.67</u> 3.30	$C_{30}H_{18}F_8O_4$	6.8 (s, 1 H, =CH); 7.6 (m, 2 H, H arom.); 8.0 (m, 4 H, H arom.); 8.5 (s, 1 H, H arom.)	44.6, 45.9 (both m, 4 F each, 2 CF ₂)	1661 (C=O)
1d	_	136—138	14	<u>55.35</u> 55.33	<u>2.73</u> 2.59	$C_{32}H_{18}F_{12}O_4$	6.75 (s, 1 H, =CH); 7.60 (m, 2 H, H arom.); 8.00 (m, 4 H, H arom.); 8.55 (s, 1 H, H arom.)	41.5 (t, 4 F, 2 CF ₂ , J = 11.0); 43.1, 43.7 (both m, 4 F each, 4 CF ₂)	1605 (C=O)
2a	174 (1)	68	17	<u>62.60</u> 62.75	<u>3.78</u> 3.95	$C_{16}H_{12}F_2O_4$	3.9 (s, 3 H, Me); 6.8 (s, 1 H, =CH); 7.6 (m, 2 H, H arom.); 7.9 (m, 4 H, H arom.); 8.5 (s, 1 H, H arom.)	37.4 (s, 2 F, CF ₂)	1770 (C=O); 1600 (C=O)
2b	180 (1)	_	22	<u>56.90</u> 57.30	<u>3.30</u> 3.37	$C_{17}H_{12}F_4O_4$	4.0 (s, 3 H, Me); 6.8 (s, 1 H, =CH); 7.6 (m, 2 H, H arom.); 7.9 (m, 4 H, H arom.); 8.5 (s, 1 H, H arom.)	41.6, 42.2 (both t, 2 F each, 2 CF ₂ , <i>J</i> = 11.0)	1776 (C=O); 1605 (C=O)
2c	160 (1)	45	20	<u>50.15</u> 50.00	<u>2.96</u> 2.63	$C_{19}H_{12}F_8O_4$	4.0 (s, 3 H, Me); 6.8 (s, 1 H, =CH); 7.6 (m, 2 H, H arom.); 7.9 (m, 4 H, H arom.); 8.5 (s, 1 H, H arom.)	40.1, 41.7 (both t, 2 F each, 2 CF ₂ , <i>J</i> = 11.0); 43.7, 42.2 (both m, 2 F each, 2 CF ₂)	1785 (C=O); 1611 (C=O)
2d	195 (1)	_	10	<u>45.51</u> 45.32	<u>2.23</u> 2.16	$C_{21}H_{12}O_4F_{12}$	4.00 (s, 3 H, Me); 6.75 (s, 1 H, =CH); 7.60 (m, 2 H, H arom.); 7.95 (m, 4 H, H arom.); 8.55 (s, 1 H, H arom.)	40.0, 41.5 (both t, 2 F each, 2 CF ₂ , <i>J</i> = 10.5); 43.2, 43.4, 43.7, 44.5 (all m, 2 F each, 4 CF ₂)	1785 (C=O); 1630 (C=O)

Experimental

¹H NMR spectra were recorded on a Bruker AC-300 spectrometer (300.13 MHz). ¹⁹F NMR spectra were measured on a Bruker WP-200 SY spectrometer (188.31 MHz). Chemical shifts (δ) are presented in ppm relative to external standards: Me₄Si (¹H) and CF₃COOH (¹⁹F). IR spectra of substances in thin layer were obtained on a UR-20 spectrometer. The physicochemical characteristics, yields of substances, elemental analysis data, and ¹H, ¹⁹F NMR and IR spectra are given in Table 5.

The luminescence-spectral properties of compounds 1a-d and 2a-d were estimated in $10^{-10}-10^{-5}$ *M* solutions of the complexones in an aqueous medium containing TOPO ($5 \cdot 10^{-5}$ mol L⁻¹) and Triton X-100 (0.1%) in the presence of Eu³⁺ ((1-5) $\cdot 10^{-5}-(1-5) \cdot 10^{-6}$ mol L⁻¹) (the ligand to ion ratio at which the 1 : 1 complex can be formed). Absorption spectra were recorded on a Perkin–Elmer M 555 spectrophotometer. Phosphorescence spectra with a time delay (t_d) of 0.05–0.10 ms relatively to the exciting pulse and a gate time (t_g) of 1.00 ms were obtained on an LS-5B spectrofluorimeter (Perkin–Elmer).

Commercial reagents and solvents, which were prepared according to known recommendations, ¹⁰ were used. Sodium methoxide was dried at 130 °C and 1 Torr prior to use.

Reaction of 2-acetylnaphthalene with dimethyl perfluoroalkanedicarboxylates (general procedure). The corresponding diester of perfluoroalkanedicarboxylic acid (30 mmol) was added with stirring for 15 min to a suspension of sodium methoxide (30 mmol) and ether (30 mL). After stirring for 30 min, 2-acetylnaphthalene (30 mmol) and ether (30 mL) were added. The reaction mixture was refluxed for 3-4 h with stirring and then cooled to 0-5 °C, and a solution of sulfuric acid (100 mmoles in 30 mL of water) was added for 10 min. The precipitate that formed was filtered off, washed with ether $(3 \times 15 \text{ mL})$ and water (3×15 mL), and dried. Tetraketones 1a-d were obtained by recrystallization from heptane, which is difficult. Therefore, pure samples of tetraketones 1a-d can be obtained already after washing of the precipitate with hot heptane. After tetraketones 1a-d were separated, the ethereal solution was washed with water (3×10 mL) and dried with sodium sulfate. Diketo esters 2a-d were obtained by fractionation *in vacuo*.

Conjugation of BSA with diketo ester 2d and estimation of labeling. A solution of diketo ester 2d (56.6 μ L, 3 · 10⁻⁷ mol L⁻¹) in ethanol was added to 0.5 mL of a solution containing BSA (1.5 · 10⁻⁸ mol L⁻¹) in a conjugating buffer (0.05 *M* solution of

sodium phosphate, 0.005 *M* solution of EDTA sodium salt, pH 8.0). After continuous stirring in the dark for 2.5 h, a mixture of products was separated by gel filtration on a column packed with Sephadex G25 (column height 10 cm, diameter 1 cm, elution rate 16 mL h⁻¹, 0.05 *M* solution of Tris buffer as eluent, pH 7.5), collecting the fractions containing protein and diketo ester **2d**.

The label to protein ratio in the conjugate was determined from the spectrophotometric characteristics of the conjugate assuming that the absorption of protein and diketo ester **2d** in the free and conjugated states remains unchanged. The following ε values were used in the calculations: for BSA, 3.3 \cdot 10⁴ and 0 L mol⁻¹ cm⁻¹; for **2d**, 7.2 \cdot 10³ and 1.36 \cdot 10⁴ L mol⁻¹ cm⁻¹ at absorption wavelengths of 278 and 333 nm, respectively.

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