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2′,3′,4′,5′-Tetraacetyl-*N*(3)-carboxymethylriboflavin derivatives: synthesis and fluorescence studies

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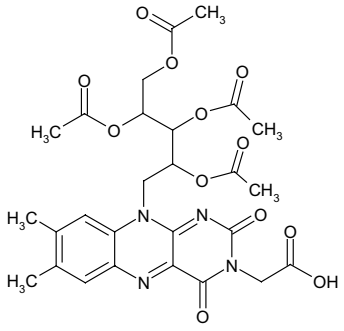
Abstract—The hitherto not described 2′,3′,4′,5′-tetraacetyl-*N*(3)-carboxymethylriboflavin (**1**) could be prepared starting from 2′,3′,4′,5′-tetraacetylriboflavin by alkylation with *tert*-butyl α-bromoacetate and benzyl α-bromoacetate, followed by deprotection reaction. The results of fluorescence studies are described.

In the course of our investigations aimed at the preparation of novel prodrugs with increased lipophilicity, the hitherto not described riboflavin derivative **1** (Fig. 1) was of interest as a novel carrier unit. This target compound is characterised by a 2′,3′,4′,5′-tetraacetylriboflavine core bearing an acetic acid spacer in position 3 of the isoalloxazin system through which drugs with amino, hydroxy or carboxy group (e.g., cyclooxygenase inhibitors) can be coupled (direct or via suitable spacer). The 2′,3′,4′,5′-tetraacetylated derivative was chosen not only due to its advantageous physicochemical properties (especially higher lipophilicity which generally increases the permeability through biological membranes) but also due to its higher (photo)chemical stability compared with riboflavin.[1] Thus, no photodegradation will occur during the synthesis of the target compounds and the fluorescence studies. It should be noted that several studies have revealed that riboflavin and derivatives with unsubstituted ribityl part undergo photodegradation under anaerobic and aerobic conditions.[2–7] Some of these photoproducts (e.g., formyllumiflavin and hydroxymethyllumiflavin) represent inhibitors of flavoenzymes or influence the riboflavin metabolism. However, since derivatisation of the sugar alcohol subunit (ribityl moiety) leads to an increase in stability, these bioactive degradation products or analogues thereof will not be built starting from 2′,3′,4′,5′-tetraacetylriboflavin.[1] Additionally, 2′,3′,4′,5′-tetraacetylriboflavin was found to exhibit no bioactivity.[4] From our ongoing studies, it has been found that the prodrugs are stable at physiological pH. Deacetylated derivatives could be detected when incubated with esterase (an enzyme which is mainly located in cells), however, in the presence of plasma (which mainly contains cholinesterase) only traces of such metabolites were formed. These results demonstrate that phototoxicity would not be a problem.

Here, we report on the synthesis of **1** and on the results of fluorescence studies. Whereas the direct carboxymethylation of 2′,3′,4′,5′-tetraacetylriboflavin (**2**) failed, reaction of **2** with the corresponding *tert*-butyl α-bromoacetate and benzyl α-bromoacetate, respectively, in the presence of potassium carbonate and catalytic amounts of sodium iodide led to **3a** and **3b** in good to excellent yields. Selective deprotection of the *tert*-butyl or benzyl



(1)

Figure 1. Structure of the target compound.

Keywords: Riboflavin derivative; Fluorescence; Prodrugs.
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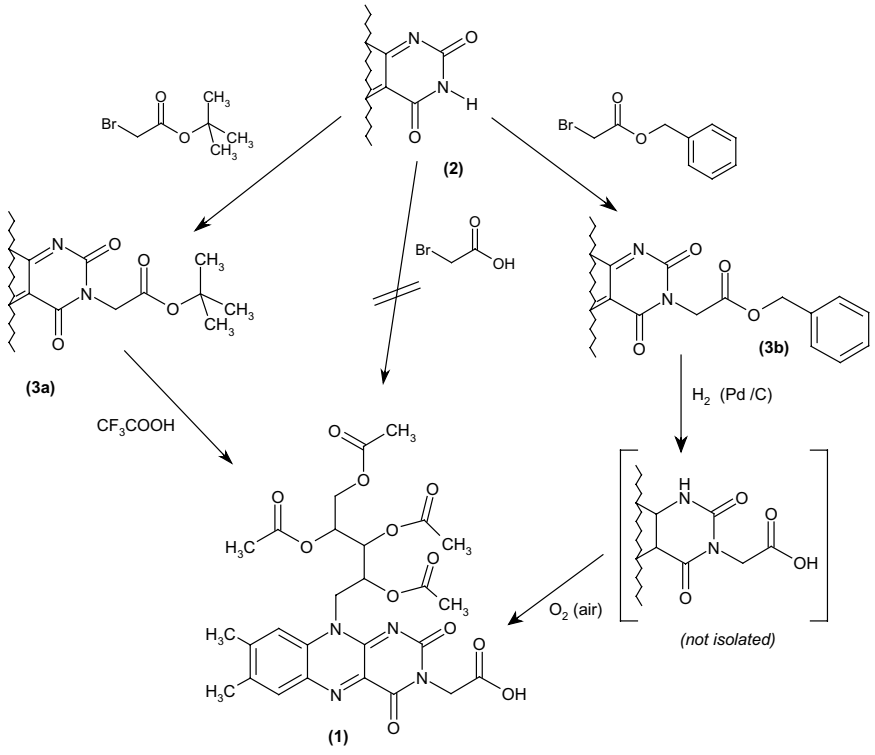
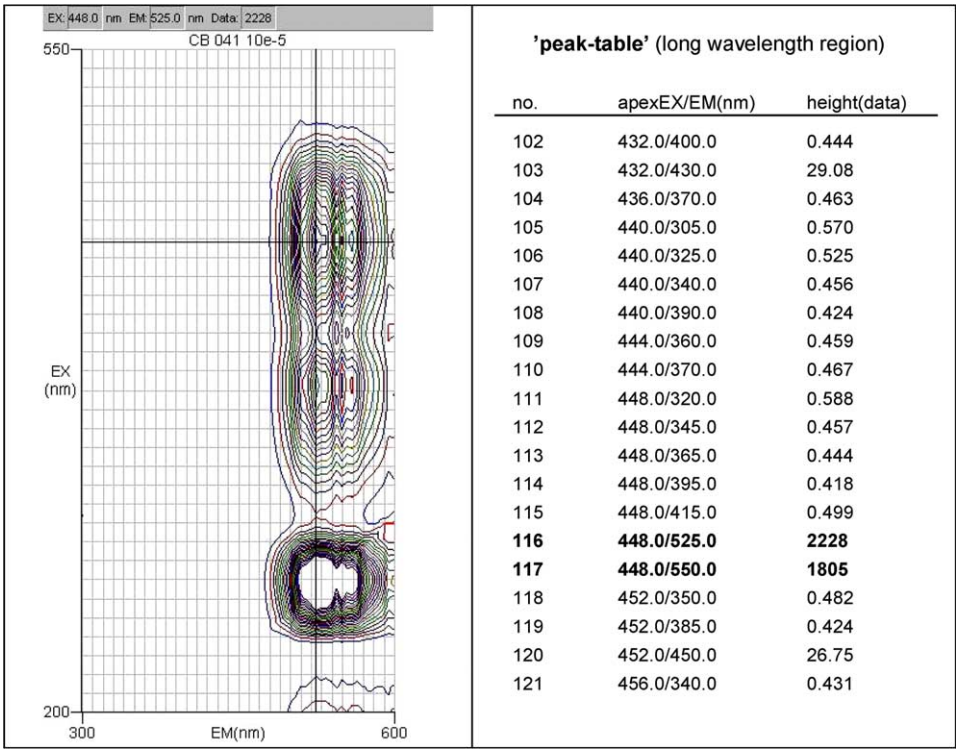


Scheme 1. Synthesis of the target compound.

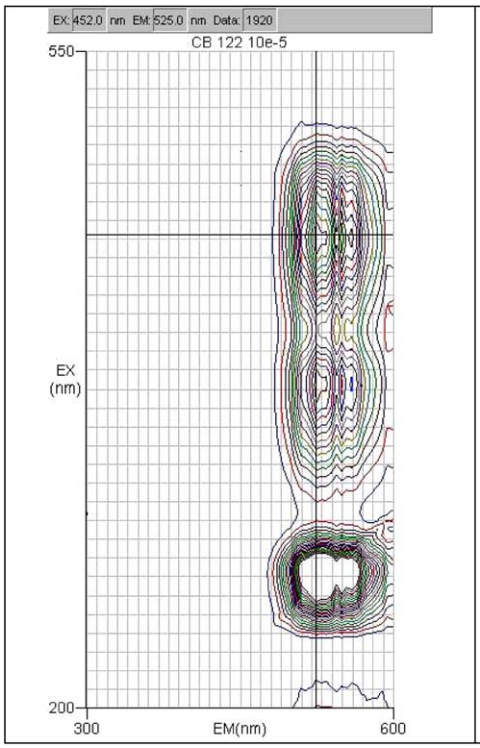


'peak-table' (long wavelength region)

no.	apexEX/EM(nm)	height(data)
102	432.0/400.0	0.444
103	432.0/430.0	29.08
104	436.0/370.0	0.463
105	440.0/305.0	0.570
106	440.0/325.0	0.525
107	440.0/340.0	0.456
108	440.0/390.0	0.424
109	444.0/360.0	0.459
110	444.0/370.0	0.467
111	448.0/320.0	0.588
112	448.0/345.0	0.457
113	448.0/365.0	0.444
114	448.0/395.0	0.418
115	448.0/415.0	0.499
116	**448.0/525.0**	**2228**
117	**448.0/550.0**	**1805**
118	452.0/350.0	0.482
119	452.0/385.0	0.424
120	452.0/450.0	26.75
121	456.0/340.0	0.431

Figure 2. 3D fluorescence spectrum of 2′,3′,4′,5′-tetraacetylriboflavin (**2**) and the corresponding peak table (for further data, see supplementary data).

ester function led to the target compound **1** (Scheme 1). Whereas the *tert*-butyl derivative **3a** was treated with trifluoroacetic acid under mild conditions, cleavage of the benzyl ester of compound **3b** was achieved by hydrogenation. It should be noted that under these conditions, the heterocyclic system was also attacked, however, the



'peak-table' (long wavelength region)

no.	apexEX/EM(nm)	height(data)
25	332.0/330.0	68.85
26	332.0/370.0	10.63
27	336.0/335.0	65.98
28	336.0/375.0	9.017
29	352.0/350.0	51.27
30	368.0/420.0	13.23
31	372.0/370.0	44.78
32	372.0/525.0	1495
33	372.0/550.0	1228
34	372.0/560.0	1118
35	384.0/440.0	14.81
36	392.0/390.0	41.54
37	396.0/395.0	38.62
38	412.0/410.0	41.46
39	416.0/415.0	35.82
40	432.0/430.0	31.19
41	452.0/450.0	29.69
42	**452.0/525.0**	**1920**
43	**452.0/550.0**	**1582**

Figure 3. 3D fluorescence spectrum of **1** and the corresponding peak table (for further data, see supplementary data).

hydrogenated tricyclic core was not isolated since it was rapidly reoxidised by contact with air. The latter could be detected by change in the colour of the reaction solution from colourless to green. Both reaction sequences can be used to get access to the target compound, however, preparation of **1** via the benzyl ester results in a higher yield and purity. The latter was verified by TLC and HPLC analysis and NMR spectroscopy (see experimental part).

The fluorescence properties of 2′,3′,4′,5′-tetraacetylriboflavin (**2**), **3a**,**b** and **1**, were investigated and compared. In contrast to the results described for 3-octylriboflavin and riboflavin itself,[6] **2** and the *N*(3)-substituted derivatives **3a**,**b** and **1** did not exhibit identical spectral characteristics. For example, there were differences in the long wavelength region, the following data were taken from the three-dimensional excitation emission spectra (see Figs. 2 and 3). It was found that a solution of 2′,3′,4′,5′-tetraacetylriboflavin (**2**) in a mixture of DMSO and phosphate buffer (pH 7.2) shows main maxima at 448 (excitation), 525, and 550 nm (emission). In the case of the *N*(3)-substituted derivatives **3a**,**b** and **1**, however, the maximum excitation has been shifted to 452 nm. These data indicate that fluorescence spectroscopy can be used to differentiate between *N*(3)-substituted and *N*(3)-unsubstituted 2′,3′,4′,5′-tetraacetylriboflavin derivatives. Thus, this technique can be used to study the potential of prodrugs in which 2′,3′,4′,5′-tetraacetylriboflavin is used as a carrier. Results concerning the preparation of such prodrugs as well as studies about their stabilities will be published later.

In summary, the *N*(3)-carboxymethylated tetraacetylriboflavine **1** which represents a novel carrier unit of potential prodrugs could be prepared. Furthermore, we have shown the possibility of using fluorescence spectroscopy as an analytical tool to differentiate between *N*(3)-substituted and *N*(3)-unsubstituted 2′,3′,4′,5′-tetraacetylriboflavin derivatives.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.05.072 (experimental section and fluorescence data).

References and notes

1. Edwards, A. M.; Bueno, C.; Saldaño, A.; Silva, E.; Kassab, K.; Polo, L.; Jori, G. *J. Photochem. Photobiol. B: Biol.* **1999**, *48*, 36–41.
2. Holström, B.; Oster, G. *J. Am. Chem. Soc.* **1961**, *83*, 1867–1871.

3. Smith, E. C.; Metzler, D. E. *J. Am. Chem. Soc.* **1963**, *85*, 3285–3288.
4. Fall, H. H.; Petering, H. G. *J. Am. Chem. Soc.* **1956**, *78*, 377–379.
5. Kuhn, R.; Wagner-Jauregg, T. *Ber. Deutsch. Chem. Ges.* **1933**, *67*, 1577–1582.
6. Ogasawara, F. K.; Wang, Y.; Bobbit, D. R. *Anal. Chem.* **1992**, *64*, 1637–1642.
7. Holzer, W.; Shirdel, J.; Zirak, P.; Penzkofer, A.; Hegemann, P.; Deutzmann, R.; Hochmuth, E. *Chem. Phys.* **2005**, *308*, 69–78.