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Synthesis and biological activities of novel dexibuprofen tetraacetylriboflavin conjugates

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Abstract—A series of novel dexibuprofen derivatives covalently linked via alkylene spacers of variable length to tetraacetylated riboflavin have been developed. The target compounds became accessible by reaction of the chloromethyl ester of dexibuprofen with tetraacetylriboflavin (compound **7**) or by synthesis of the appropriate *N*3-(ω-iodoalkyl)-2′,3′,4′,5′-tetraacetylriboflavin followed by treatment with dexibuprofen (derivatives **8**–**11**), respectively. Biological screening revealed that the target compounds exhibit antiproliferative effects on MCF-7 breast cancer and HT-29 colon carcinoma cells with IC_{50} values in the range of 8–15 μM. Enzymatic studies on human platelets indicated significant COX-1 inhibitory activities of the target compounds.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a widely used class of therapeutic agents. Their main mode of action is the inhibition of the cyclooxygenase enzymes (i.e., COX-1 and COX-2) leading to a reduction in the synthesis of prostaglandins, the messenger molecules in the process of inflammation. In recent years, several epidemiological, clinical, and experimental studies have shown that NSAIDs also exhibit anticancer properties.[1] Moreover, it was demonstrated that long-term use of NSAIDs reduces the recurrence risk in various malignancies such as breast and colon cancer significantly.[2–5] Despite showing good chemoprotective and cytostatic in vivo activity, the NSAIDs only exhibit good cell activity at high concentrations.[6–11]

In this context, it should be noted so far that the mechanism for the antitumor activity of NSAIDs is still unknown. However, some hypotheses have been proposed. Several groups have postulated that both, anti-inflammatory activities and anticancer effects are due to a reduction of products formed by cyclooxygenase (COX) or lipoxygenase (LOX) catalyzed reactions.[12–14]

On the contrary, a recent study demonstrates that the antiproliferative effects of *R*- and *S*-ibuprofen do not depend on COX activity.[15] Thus, additional studies will be necessary to understand the molecular mechanism of action. In spite of the positive findings, long-term use of non-selective NSAIDs in this area might be limited by adverse gastrointestinal effects ranging from dyspepsia to serious complications such as bleeding or perforated gastroduodenal ulcers. The risk of gastrointestinal complications results from decreased synthesis of gastrointestinal protective prostaglandins based on COX-1 inhibition.[16,17] Additionally, classical NSAIDs can cause local toxicity due to their acidic functionality[17] which is required for proper receptor interaction and inhibition.[18] Consequently, there are two main possibilities to lower the gastrointestinal toxicity. Though selective COX-2 inhibitors can significantly lower incidence of gastrointestinal toxicity, however, their use is associated with increased risk of cardiovascular events. An alternative approach to solve the problem of the ulcerogenic properties of NSAIDs consists in masking the acidic function.[19–22]

The interesting activities mentioned above prompted us to investigate novel NSAID derivatives with protected carboxylic acid function. Here we report on ester derivatives of dexibuprofen which was chosen for this concept as it showed promising long-term results in retrospective clinical studies and in animals. It should

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be noted that this compound exhibits only low cell growth inhibitory activity in vitro.[2,23] The minor cell growth inhibitory activity may be attributed to a low cellular uptake under in vitro conditions. We chose dexibuprofen since this pure enantiomer has been reported to be more effective and better tolerated in long-term studies than racemic ibuprofen.[24]

In our target compounds, the ibuprofen moiety is linked via different spacer units to a riboflavin derivative. The latter moiety was chosen due to the fascinating biological activities described in the literature, especially protection against oxidant-mediated inflammatory organ injury,[25] reduction of cancer chemotherapy toxicity,[26] and antiinflammation.[27] 2′,3′,4′,5′-Tetraacetylriboflavin (TAR) was selected for reasons of good photostability and solubility.[28]

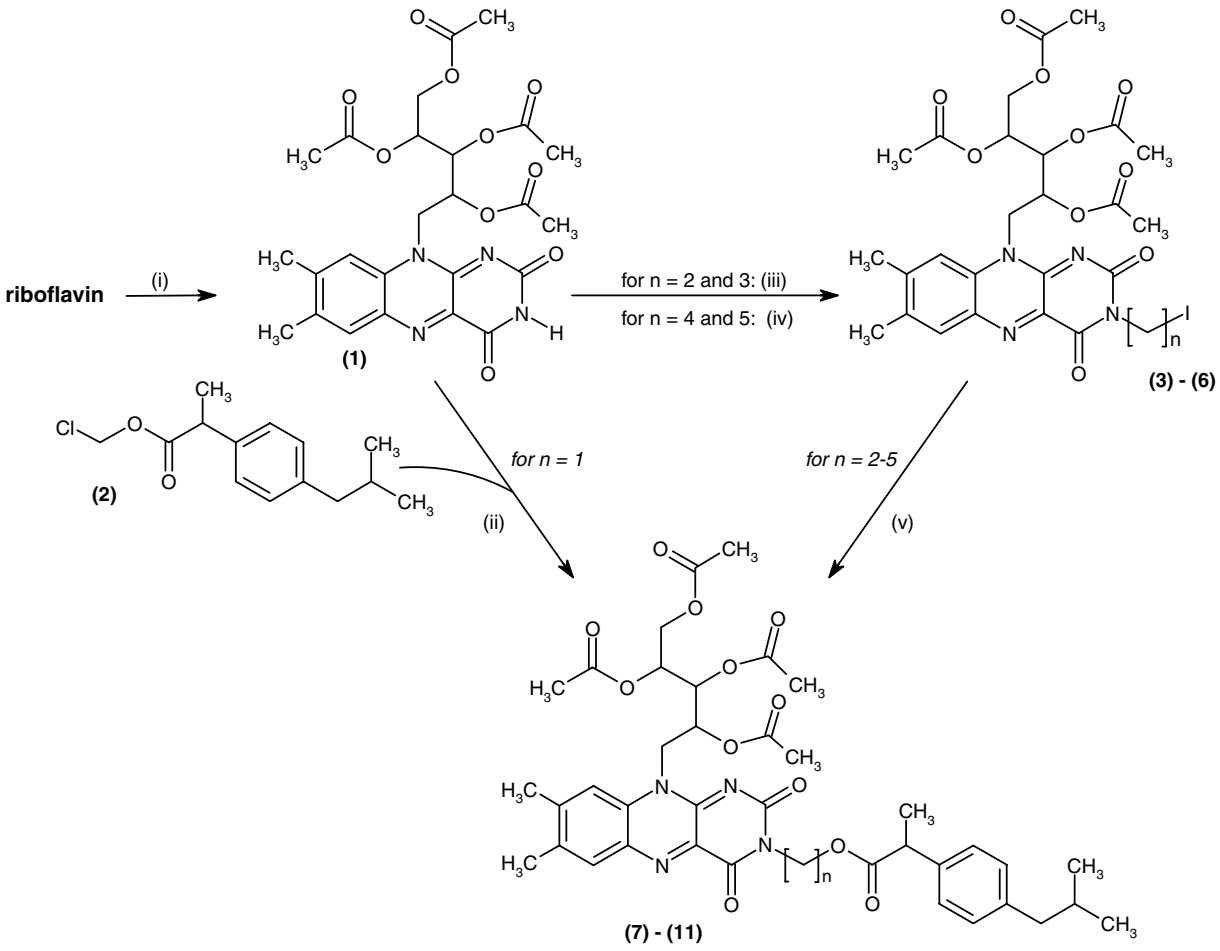
The target compounds **7**–**11** were synthesized as depicted in Scheme 1. Initially, treatment of riboflavin with acetic acid anhydride and pyridine allowed us to obtain the already known 2′,3′,4′,5′-tetraacetylriboflavin (**1**)[29] in gram amounts. This derivative was used as a key intermediate for the preparation of the target compounds.

In the case of the envisaged **7** which is characterized by a methylene spacer between the TAR carrier and the NSAID, compound **2**[30] was considered as a suitable synthon. This chloromethyl ester of dexibuprofen (**2**) was available in a two-step reaction using bromochlorome-

Table 1. Antiproliferative activities expressed as IC_{50} value (μM) ± standard error[a]

Compound	HT-29 cells	MCF-7 cells
Dexibuprofen	>1000	795 (±119)
7	12.1 (±0.8)	12.3 (±0.0)
8	12.7 (±0.8)	14.9 (±1.1)
9	9.3 (±0.6)	7.8 (±2.2)
10	10.5 (±0.8)	9.5 (±1.6)
11	13.1 (±0.6)	9.5 (±1.4)
3a	>100	>100
1	90.9 (±0.8)	53.4 (±3.5)
5-FU	7.3 (±1.0)	4.8[34]

[a] Data obtained from two independent experiments.



Scheme 1. Synthesis of the target compounds **7**–**11**. Reagents and conditions: (i) Ac_2O, pyridine, reflux; (ii) $N(C_2H_5)_3$, NaI (cat.) in acetone/CH_2Cl_2, reflux; (iii) 1. HO–$(CH_2)_n$–X with X = I (n = 2) or Br (n = 3), K_2CO_3 in *N*,*N*-dimethylformamide, room temperature; 2. CH_3SO_2Cl, pyridine in CH_2Cl_2, room temperature; 3. NaI in dry acetone, reflux; (iv) $(CH_2)_nI_2$, K_2CO_3 in *N*,*N*-dimethylformamide, room temperature; (v) dexibuprofen, $N(C_2H_5)_3$, Na_2SO_4 in acetone, reflux.

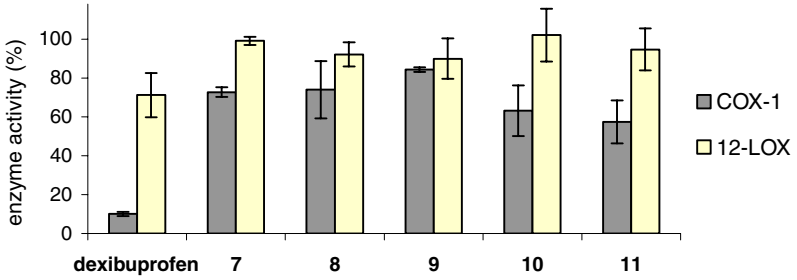


Figure 1. Enzyme activities after incubation with the test compounds (10 μM) (results obtained from 3 to 5 independent experiments).

thane and chlorosulfonic acid followed by reaction with the NSAID in a two-phase system in the presence of tetrabutylammonium hydrogensulfate as the phase-transfer catalyst. Finally, the target conjugate **7** resulted from base-mediated reaction of **2** with **1** in a mixture of acetone and dichloromethane as the solvent. The crude reaction product was purified by column chromatography to give a yellow oil which solidified in vacuo.

For the synthesis of **8**–**11**, alternative reaction sequences were employed. It was planned to introduce an appropriate ω-iodo substituted alkylene spacer into the 2′,3′,4′,5′-tetraacetylriboflavin. Whereas employment of α,ω-diiodobutane and α,ω-diiodopentane led to the desired ω-iodoalkylated derivatives **5** and **6**, synthesis of the corresponding iodoethyl and iodopropyl derivatives failed. The latter were accessible by initial preparation of the ω-hydroxyalkyl-substituted carrier units **3a** and **4a**, followed by conversion to the appropriate mesylates **3b** and **4b**, and subsequent treatment of the latter with sodium iodide. The desired compounds **8**–**11** were prepared starting from the iodoalkyl derivatives **3**–**6** and dexibuprofen in the presence of triethylamine. All compounds were characterized by ^{1}H NMR and IR spectroscopy as well as by mass spectrometry and elemental analysis.[31]

For the proliferation experiments MCF-7 breast cancer and HT-29 colon cancer cells were selected since these types of tumors have been reported to exhibit high sensitivity to NSAID treatments.[12,13,32,33] In addition to the novel conjugates **7**–**11**, dexibuprofen, tetraacetylriboflavin (**1**), *N*3-(2-hydroxyethyl)-TAR (**3a**) as well as the established cytostatic agent 5-fluorouracil (5-FU) were used as additional references. IC_{50} values for these compounds were determined and are presented in Table 1.

Interestingly, our conjugates **7**–**11** displayed IC_{50} values in the range of 7.8–14.9 μM in both cell lines. Therefore, these novel derivatives are of comparable activity with 5-FU which is widely employed in the treatment of cancer. By contrast, the main components of **7**–**11** [i.e., dexibuprofen and TAR (**1**)] as well as the potential decomposition product **3a** showed weak or no antiproliferative activity.

These findings could be interpreted in different ways: on the one hand, the cytotoxicity of the target compounds **7**–**11** might be the result of COX inhibition. Since it is known that an acidic substructure is essential for COX inhibition[35] and initial studies showed that the conjugates were chemically stable (determined by incubation in phosphate buffer, data not shown), the activities of **7**–**11** may be explained with an intracellular release of dexibuprofen. Thus, the uptake into the cells and subsequent enzymatic ester hydrolysis should be critical parameters. Another explanation for the elevated cytotoxicity of **7**–**11** is that these conjugates are able to act not only as drug delivery agents but also as independent bioactive compounds. In either case pharmacokinetic parameters must be taken into account.

In order to gain additional insight into the mode of action, we evaluated the inhibitory effects of **7**–**11** on COX-1 and 12-LOX using an established human platelet assay which quantifies the eicosanoids 12(*S*)-hydroxy-5-*cis*-8, 10-*trans*-heptadecatrienoic acid (i.e., 12-HHT; COX-1 product) and 12(*S*)-hydroxy-5,8-*cis*-10-*trans*-14-*cis*-eicosatetraenoic acid (i.e., 12-HETE; 12-LOX product), respectively.[36,37]

Under the test conditions (i.e., incubation for 60 min in buffer solution at 37 °C), all novel dexibuprofen tetraacetylriboflavin conjugates (**7**–**11**) lowered the activity of COX-1 (see Fig. 1). However, none of them reached the potency of dexibuprofen (90% inhibition) which was used as a reference. While **7**–**9** lowered the eicosanoid levels by approximately 15–30%, the compounds **10** and **11** were more active (37% and 43% inhibition, respectively). These results indicate the uptake of compounds **7**–**11** into the platelets and the intracellular release of ibuprofen which may also take place in the case of the tumor cells MCF-7 and HT-29. Moreover, our experiments indicated that the enzyme 12-LOX is inhibited by dexibuprofen (29% inhibition), while **7**–**11** did not influence 12-LOX catalyzed eicosanoid formation significantly (inhibition lower than 11% in all cases). Additional investigations to get insight into the mechanism of action as well as into structure–activity relationships are in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.10.087.

References and notes

1. Marx, J. *Science* **2004**, *306*, 966.
2. Yao, M.; Zhou, W.; Sangha, S.; Albert, A.; Chang, A. J.; Liu, T. C.; Wolfe, M. M. *Clin. Cancer Res.* **2005**, *11*, 1618.
3. Sheng, H.; Shao, J.; Kirkland, S. C.; Isakson, P.; Coffey, R. J.; Morrow, J.; Beauchamp, R. D.; DuBois, R. N. *J. Clin. Invest.* **1997**, *99*, 2254.
4. Coruzzi, G.; Menozzi, A.; Dobrilla, G. *Curr. Drug Targets Inflamm. Allergy* **2004**, *3*, 43.
5. Ulrich, C. M.; Bigler, J.; Potter, J. D. *Nat. Rev. Cancer* **2006**, *6*, 130.
6. Sotiriou, C.; Lacroix, M.; Lagneaux, L.; Berchem, G.; Body, J. J. *Anticancer Res.* **1999**, *19*, 2997.
7. Rodriguez-Burford, C.; Barnes, M. N.; Oelschlager, D. K.; Myers, R. B.; Talley, L. I.; Partridge, E. E.; Grizzle, W. E. *Clin. Cancer Res.* **2002**, *8*, 202.
8. Fulton, A. M. *Int. J. Cancer* **1984**, *33*, 375.
9. Cunningham, D. C.; Harrison, L. Y.; Shultz, T. D. *Anticancer Res.* **1997**, *17*, 197.
10. Waskewich, C.; Blumenthal, R. D.; Li, H.; Stein, R.; Goldenberg, D. M.; Burton, J. *Cancer Res.* **2002**, *62*, 2029.
11. Sanchez-Alcazar, J. A.; Bradbury, D. A.; Pang, L.; Knox, A. J. *Lung Cancer* **2003**, *40*, 33.
12. Mazhar, D.; Ang, R.; Waxman, J. *Br. J. Cancer* **2006**, *94*, 346.
13. Giardello, F. M.; Offerhaus, G. J. A.; DuBois, R. N. *Eur. J. Cancer* **1995**, *31A*, 1071.
14. Shureiqi, I.; Lippman, S. M. *Cancer Res.* **2001**, *61*, 6307.
15. Janssen, A.; Maier, T. J.; Schiffmann, S.; Coste, O.; Seegel, M.; Geisslinger, G.; Grösch, S. *Eur. J. Pharmacol.* **2006**, *540*, 24.
16. Russell, R. I. *Postgrad. Med. J.* **2001**, *77*, 82.
17. Becker, J. C.; Domschke, W.; Pohle, T. *Br. J. Clin. Pharmacol.* **2004**, *58*, 587.
18. Vane, J. R.; Bakhle, Y. S.; Botting, R. M. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97.
19. Galanakis, D.; Kourounakis, A. P.; Tsiakitzis, K. C.; Doulgkeris, C.; Rekka, E. A.; Gavalas, A.; Kravaritou, C.; Charitos, C.; Kourounakis, P. N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3639.
20. Salvatella, M.; Rossi, I.; Del Valle, J. C.; Gutierrez, Y.; Pereda, C.; Samper, B.; Feliu, J. E. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2004**, *286*, G711.
21. Ogiso, T.; Iwaki, M.; Tanino, T.; Nagai, T.; Ueda, Y.; Muraoka, O.; Tanabe, G. *Biol. Pharm. Bull.* **1996**, *19*, 1178.
22. Bansal, A. K.; Dubey, R.; Khar, R. K. *Drug Dev. Ind. Pharm.* **1994**, *20*, 2025.
23. Robertson, F. M.; Parrett, M. L.; Joarder, F. S.; Ross, M.; Abou-Issa, H. M.; Alshafie, G.; Harris, R. E. *Cancer Lett.* **1998**, *122*, 165.
24. Bonabello, A.; Galmozzi, M. R.; Canapaio, R.; Isaia, G. C.; Serpe, L.; Muntoni, E.; Zara, G. P. *Anesth. Analg.* **2003**, *97*, 402.
25. Seekamp, A.; Hultquist, D. E.; Till, G. O. *Inflammation* **1999**, *23*, 449.
26. Burzynski, S. R. **2003**, WO 2003045372 A1.
27. Granados-Soto, V.; Terán-Rosales, F.; Rocha-González, H. I.; Reyes-Garcia, G.; Medina-Santillán, R.; Rodriguez-Silverio, J.; Flores-Murrieta, F. J. *Eur. J. Pharmacol.* **2004**, *492*, 35.
28. Edwards, A. M.; Bueno, C.; Saldano, A.; Silva, E.; Kassab, K.; Polo, L.; Jori, G. *J. Photochem. Photobiol. B* **1999**, *48*, 36.
29. Kuhn, R.; Wagner-Jauregg, Th. *Ber. Dtsch. Chem. Ges.* **1933**, *67*, 1577.
30. Loubinoux, B.; Colin, J. L.; Thomas, V. *Eur. J. Med. Chem.* **1991**, *26*, 461.
31. General procedures for the synthesis of the target compounds **7**–**11**: *Synthesis of compound* (**7**): A solution of 3.5 mmol of the chloromethylester of dexibuprofen (**2**) in 5 mL of dry acetone was added to a stirred cold (ice bath) mixture of **1** (0.545 g, 1.0 mmol), triethylamine (1.0 mmol), anhydrous Na_2SO_4 (0.03 g), and catalytic amounts of NaI in 3 mL of a mixture of acetone and dichloromethane (3/2), and treated as described below. *Synthesis of compounds* (**8**–**11**): A solution of dexibuprofen (3.0 equiv, 1.3–2.0 mmol) and triethylamine (3.0 equiv) in 1.0–2.5 mL of dry acetone was added to a stirred cold (ice bath) mixture of 1.0 equiv (0.43–0.68 mmol) of the appropriate *N*3-(ω-iodoalkyl)-substituted 2′,3′,4′,5′-tetra-acetylriboflavin (**3**–**6**) and 0.03 g anhydrous Na_2SO_4 in 1.5–2.5 mL of dry acetone. Then, the appropriate reaction mixture was slowly warmed to ambient temperature by removing the ice bath and the mixture was stirred for 1 h at room temperature. The reaction temperature was then increased to 50 °C (**7**) or 60 °C (**8**–**11**), respectively, until the alkylating reagent was completely consumed (TLC monitoring: dichloromethane:ethyl acetate = 2/1). Then, the reaction mixture was diluted with 20 mL of dichloromethane, the organic phase was washed with water, saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated. The crude residue was purified by column and/or circular chromatography to give the appropriate target compound which was taken up in one milliliter of a 1:1 mixture of dichloromethane and diisopropyl ether. Finally, the organic solvents were slowly evaporated to give a foamy solid which could be scrapped out of the flask.
2′,3′,4′,5′-Tetraacetyl-3-{[1-(4-isobutylphenyl)ethyl]carbonyloxymethyl}riboflavin (**7**): Column chromatography (dichloromethane:ethyl acetate = 2/1) yielded 0.110 g (14.4%) of a yellow product. IR (KBr) 1748 cm^{-1}; MS *m*/*z* 763 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ 8.03 (s, 1H, 9-H), 7.55 (s, 1H, 6-H), 7.19 (d, J = 8.2 Hz, 2H, phenyl-H), 7.05 (d, J = 8.2 Hz, 2H, phenyl-H), 6.14, 6.09 (both d with J = 9.3 Hz, 2H, N–CH_2–O), 5.72–5.61 (m, 1H, ribityl-CH), 5.49–5.36 (m, 2H, 2× ribityl-CH), 5.26–4.69 (m, 2H, 2× ribityl-CH), 4.44 (dd, J = 2.6 Hz, J = 12.4 Hz, 1H, ribityl-CH), 4.24 (dd, J = 5.3 Hz, J = 12.3 Hz, 1H, ribityl-CH), 3.77–3.65 (m, 1H, –C*H*Me), 2.56 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.41 (d, J = 7.4 Hz, 2H, –CH_2–$CHMe_2$), 2.28 (s, 3H, $COCH_3$), 2.22 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.91–1.69 (m, 1 H, –CH_2–C*H*Me_2), 1.74 (s, 3H, $COCH_3$), 1.49(d, J = 7.0 Hz, 3H, –CHCH_3), 0.87 (d, J = 6.6 Hz, 6H, –CH_2–$CH(CH_3)_2$). Anal. ($C_{39}H_{46}N_4O_{12}$) C, H, N.
2′,3′,4′,5′-Tetraacetyl-3-{2-{[1-(4-isobutylphenyl)ethyl]carbonyloxy}ethyl}riboflavin (**8**): Column chromatography (dichloromethane:ethyl acetate = 2/1 followed by ethyl acetate:diethyl ether = 2/1) yielded 0.060 g (26%) of a yellow product. IR (KBr) 1748 cm^{-1}; MS *m*/*z* 777 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ 8.06 (s, 1H, 9-H), 7.55 (s, 1H, 6-H), 7.16 (d, J = 8.1 Hz, 2H, phenyl-H), 6.99 (d, J = 8.1 Hz, 2H, phenyl-H), 5.73–5.60 (m, 1H, ribityl-CH), 5.49–5.37 (m, 2H, 2× ribityl-CH), 5.27–4.63 (m, 2H, 2× ribityl-CH), 4.58–4.20 (m, 6H, N–CH_2, –COOCH_2, 2× ribityl-CH), 3.67 (q, J = 7.1 Hz, 1H, –C*H*Me), 2.56 (s, 3 H, CH_3), 2.45

(s, 3H, CH_3), 2.33 (d, J = 7.2 Hz, 2H, –CH_2–CHMe$_2$), 2.30 (s, 3H, $COCH_3$), 2.22 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.82–1.69 (m, 1H, –CHMe$_2$), 1.75 (s, 3H, $COCH_3$), 1.45 (d, J = 7.1 Hz, 3H, –CHCH_3), 0.84 (d, J = 6.6 Hz, 6H, –CH$_2$–CH(CH_3)$_2$). Anal. ($C_{40}H_{48}N_4O_{12}$) C, H, N.

2′,3′,4′,5′-Tetraacetyl-3-{3-{[1-(4-isobutylphenyl)ethyl]carbonyloxy}propyl}riboflavin (**9**): Column chromatography (dichloromethane:ethyl acetate = 2/1 followed by ethyl acetate:diethyl ether = 2/1) yielded 0.140 g (35%) of a yellow solid. IR (KBr) 1751 cm^{-1}; MS m/z 791 (M+1)$^+$. ^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1H, 9-H), 7.54 (s, 1H, 6-H), 7.22 (d, J = 8.2 Hz, 2H, phenyl-H), 7.07 (d, J = 8.2 Hz, 2H, phenyl-H), 5.74–5.60 (m, 1H, ribityl-CH), 5.50–5.37 (m, 2H, 2× ribityl-CH), 5.22–4.71 (m, 2H, 2× ribityl-CH), 4.44 (dd, J = 2.5 Hz, J = 12.3 Hz, 1H, ribityl-CH), 4.28–4.07 (m, 5H, N–CH_2, –COOCH_2, ribityl-CH), 3.72 (q, J = 7.2 Hz, 1H, –CHMe), 2.55 (s, 3H, CH_3), 2.44–2.40 (m, 5H, CH_3, –CH_2–CHMe$_2$), 2.29 (s, 3H, $COCH_3$), 2.22 (s, 3H, $COCH_3$), 2.11–1.69 (m, 3H, –CH_2–, CH$_2$–CHMe$_2$), 2.07 (s, 3H, $COCH_3$), 1.73 (s, 3H, $COCH_3$), 1.50 (d, J = 7.2 Hz, 3H, –CHCH_3), 0.88(d, J = 6.4 Hz, 6H, –CH$_2$–CH(CH_3)$_2$). Anal. ($C_{41}H_{50}N_4O_{12}$) C, H, N.

2′,3′,4′,5′-Tetraacetyl-3-{4-{[1-(4-isobutylphenyl)ethyl]carbonyloxy}butyl}riboflavin (**10**): Column chromatography (dichloromethane:ethyl acetate = 2/1 followed by ethyl acetate:diethyl ether = 2/1) yielded 0.280 g (51%) of a yellow compound. IR (KBr) 1752 cm^{-1}; MS m/z 805 (M+1)$^+$. ^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1H, 9-H), 7.53 (s, 1H, 6-H), 7.20 (d, J = 8.3 Hz, 2H, phenyl-H), 7.07 (d, J = 8.3 Hz, 2H, phenyl-H), 5.73–5.62 (m, 1H, ribityl-CH), 5.50–5.37 (m, 2H, 2× ribityl-CH), 5.24–4.68 (m, 2H, 2× ribityl-CH), 4.44 (dd, J = 2.4 Hz, J = 12.2 Hz, 1H, ribityl-CH), 4.24–4.03 (m, 5H, N–CH_2, –COOCH_2, ribityl-CH), 3.68 (q, J = 7.2 Hz, 1 H, –CHMe), 2.55 (s, 3H, CH_3), 2.44–2.40 (m, 5H, CH_3, –CH_2–CHMe$_2$), 2.29 (s, 3H, $COCH_3$), 2.22 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.93–1.64 (m, 5H, 2× –CH_2, –CH$_2$–CHMe$_2$), 1.73 (s, 3H, $COCH_3$), 1.48 (d, J = 7.2 Hz, 3H, –CHCH_3), 0.87 (d, J = 6.8 Hz, 6H, –CH$_2$–CH(CH_3)$_2$). Anal. ($C_{42}H_{52}N_4O_{12} \times 0.2$ diisopropyl ether $\times$ 0.3 H_2O) C, H, N.

2′,3′,4′,5′-Tetraacetyl-3-{5-{[1-(4-isobutylphenyl)ethyl]carbonyloxy}pentyl}riboflavin (**11**): Column chromatography (dichloromethane:ethyl acetate = 2/1) and circular chromatography (dichloromethane:ethyl acetate = 2/1) yielded 0.320 g (59%) of a yellow solid; IR (KBr) 1752 cm^{-1}; MS m/z 819 (M+1)$^+$. ^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1H, 9-H), 7.53 (s, 1H, 6-H), 7.20 (d, J = 8.2 Hz, 2H, phenyl-H), 7.09 (d, J = 8.2 Hz, 2H, phenyl-H), 5.71–5.63 (m, 1H, ribityl-CH), 5.46–5.37 (m, 2H, 2× ribityl-CH), 5.27–4.67 (m, 2H, 2× ribityl-CH), 4.44 (dd, J = 2.5 Hz, J = 12.3 Hz, 1H, ribityl-CH), 4.24 (dd, J = 5.3 Hz, J = 12.5 Hz, 1H, ribityl-CH), 4.11–3.96 (m, 4H, N–CH_2, COOCH_2), 3.68 (q, J = 7.1 Hz, 1H, –CHMe), 2.55 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.44 (d, J = 7.2 Hz, 2H, –CH_2–CHMe$_2$), 2.29 (s, 3H, $COCH_3$), 2.22 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 1.91–1.58 (m, 5H, 2× –CH_2–, –CH$_2$–CHMe$_2$), 1.73 (s, 3H, $COCH_3$), 1.50–1.26 (m, 2H, –CH_2), 1.48 (d, J = 7.1 Hz, 3H, –CHCH_3), 0.89 (d, J = 6.6 Hz, 6H, –CH$_2$–CH(CH_3)$_2$). Anal. ($C_{43}H_{54}N_4O_{12} \times 0.4$ diisopropyl ether $\times$ 0.4 H_2O) C, H, N.

32. Bange, J.; Zwick, E.; Ullrich, A. *Nat. Med.* **2001**, *7*, 548.
33. Harris, R. E.; Chlebowski, R. T.; Jackson, R. D.; Frid, D. J.; Ascenseo, J. L.; Anderson, G.; Loar, A.; Rodabough, R. J.; White, E.; McTiernan, A. *Cancer Res.* **2003**, *63*, 6096.
34. Ott, I.; Schmidt, K.; Kircher, B.; Schumacher, P.; Wiglenda, T.; Gust, R. *J. Med. Chem.* **2005**, *48*, 622.
35. Mancini, J. A.; Riendeau, D.; Falgueyret, J. P.; Vickers, P. J.; O'Neill, G. P. *J. Biol. Chem.* **1995**, *270*, 29372.
36. Albert, D.; Zündorf, I.; Dingermann, T.; Müller, W. E.; Steinhilber, D.; Werz, O. *Biochem. Pharmacol.* **2002**, *64*, 1767.
37. Ott, I.; Koch, T.; Shorafa, H.; Bai, Z.; Poeckel, D.; Steinhilber, D.; Gust, R. *Org. Biomol. Chem.* **2005**, *3*, 2282.