Synthesis of Flavin–Calix[4]arene Conjugate Derivatives

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The synthesis of two new flavin substituted calix[4]arene derivatives, **9** and **10**, is described. The first flavin substituted calix[4]arene derivative **9** was synthesized by the reaction of 3-methylalloxazine (**5**) with 25,27-bis(3-bromopropoxy)-26,28-dihydroxy-5,11,17,23-tetra(*tert*-butyl)calix[4]arene (**4**) in high yield (92%). The other derivative **10** was prepared from 3-methylalloxazine-1-acetic acid (**7**) and 25,27-bis(3-cyanopropoxy)calix[4]arene (**3**). All new compounds were characterized by a combination of FT-IR and ^{1}H-NMR spectroscopy, and elemental-analysis techniques.

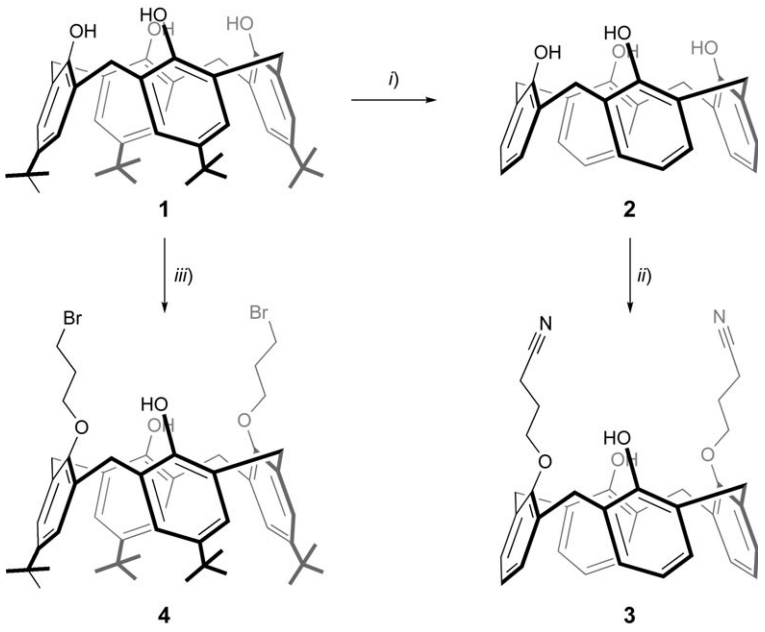
Introduction. – Flavins play an important role in living organisms [1], and they are involved in several important photobiological and photochemical processes, such as phototropism, phototaxis, and photodynamic action [2][3]. Since 1966, the photochemistry and photophysics of alloxazine derivatives have been studied, due to the discovery of the proton-transfer reactions in lumichrome and related compounds [4][5]. It is well-known that substituted alloxazine (= benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) derivatives, mainly lumichromes, are present in many foods and formed in the normal metabolic process of ingested riboflavin [6]. Recently, alloxazines have attracted attention due to realization of their possible involvement in a wide variety of biological systems [6][7]. For instance, it has been shown that lumichrome may be used to inhibit flavin reductase in living *Escherichia coli* cells [8].

Supramolecular science has been developed tremendously over the past 30 years [9][10]. It is known that preorganization and cooperativeness of multifunctional groups play a major role in biological reaction kinetics [11]. Some calix[4]arene derivatives, which bear azo groups [12], guadinium units [13], or nucleoside hybrids [11], are compounds which can be used as enzymes in the cell. At the same time, flavins that are obtained by condensation between α-phenylenediamine and alloxane [14] are involved in several biological processes [15]. These are generally redox cofactors involved in electron redox processes [16] of enzymatic reactions [17][18]. To this end, we considered that compounds containing both flavin and calixarene units, *i.e.*, **9** and **10**, respectively, might have interesting biological activities. Here, we report the first synthesis of calixarene derivatives that contain flavin units.

Results and Discussion. – *Synthesis.* The calix[4]arenes can be functionalized with desired groups both at the upper and the lower rim [19][20] in order to obtain appropriate arrangements or to achieve the preorganized conformation [9].

The aim of this work was the design and synthesis of new calix[4]arene derivatives, which are cited as flavin–conjugate calixarene derivatives. All new compounds were characterized by means of ^{1}H-NMR and IR spectroscopy, and elemental analyses. The synthetic routes leading to new calixarene derivatives are depicted in *Schemes 1* and *2*.

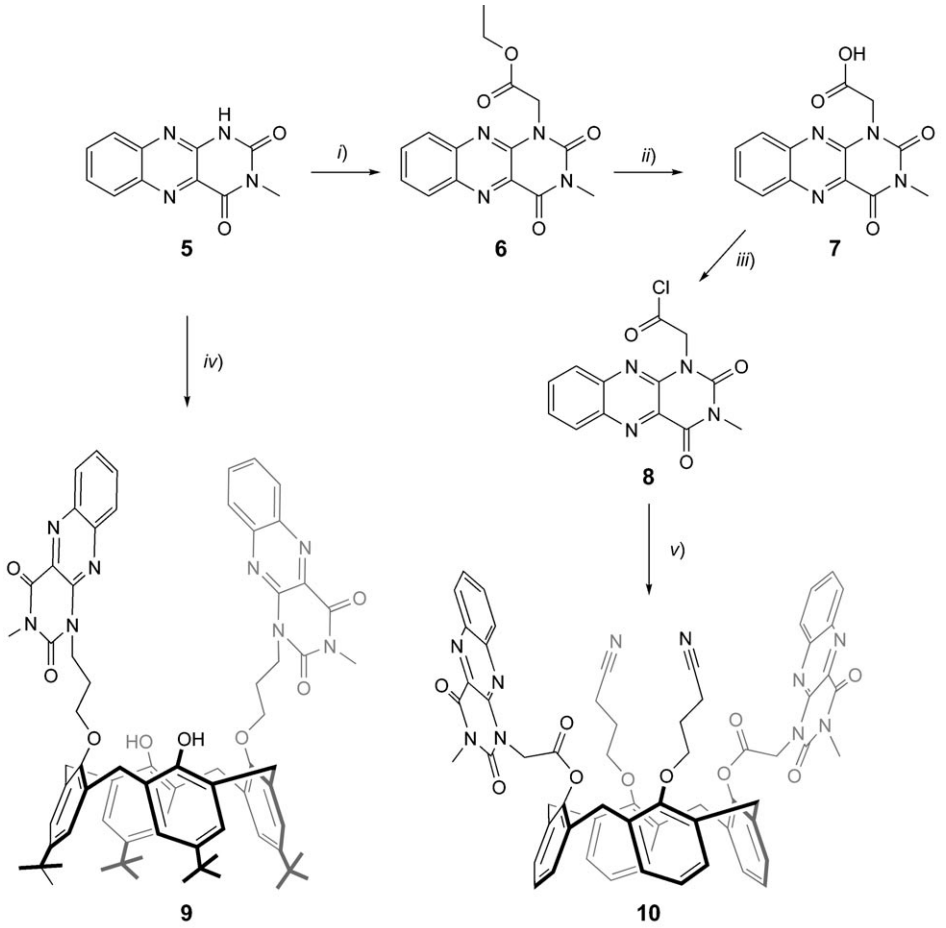
Scheme 1. *The Synthetic Route for the Preparation of* **3** *and* **4**



i) $AlCl_3$, PhOH, toluene, r.t., 78%. *ii*) K_2CO_3, MeCN, $Cl(CH_2)_3CN$, reflux, 79%. *iii*) 1,3-Dibromopropane, K_2CO_3, MeCN, reflux, 64%.

p-(*tert*-Butyl)calix[4]arene **1** was chosen as the starting material, and it was transformed to the derivatives **3** and **4** according to known procedures [19] [21] [22] (see *Scheme 1*). The flavin part of the molecules was synthesized starting from compound **5**, which is available by condensation of benzene-1,2-diamine with *N*-methylalloxane according to the procedure described in [14]. Treatment of **5** with $BrCH_2COOEt$ afforded ethyl 3-methylalloxazine-1-acetate (**6**; *Scheme 2*). Then, upon hydrolysis of **6** with HCl, the flavin-carboxylic acid **7** was obtained. Treatment of **7** with $SOCl_2$ at 45° for 2 h yielded the Cl derivative **8**. The substitution of *p*-(*tert*-butyl)calix[4]arene derivative (**4**) at its bromoalkoxy chains was conducted in the presence of K_2CO_3 in DMF under N_2 with flavin to afford the cone conformer flavin–calix[4]arene conjugate **9** in high yield (92%). The other flavin–calix[4]arene conjugate **10** was synthesized by treatment of 1-(2-chloro-2-oxoethyl)-3-methylalloxazine (**8**) with 25,27-bis(3-cyanopropoxy)calix[4]arene (**3**) in MeCN in the presence of K_2CO_3 at 85° for 37 h in 42% yield. The ^{1}H-NMR spectra of **9** and **10** display a typical *AX* pattern for the H-atoms of a CH_2 bridge (ArCH_2Ar) of the calixarene moiety at δ(H) 4.17 and 3.5 ppm (J = 12.8)

Scheme 2. *The Synthetic Route for the Preparation of* **9** *and* **10**



i) $BrCH_2COOEt$, K_2CO_3, DMF, r.t., 77%. *ii*) HCl, 80–90°, 55%. *iii*) $SOCl_2$, 45°. *iv*) **4**, NaI, K_2CO_3, DMF, N_2, r.t., 92%. *v*) **3**, MeCN, K_2CO_3, 85°, 42%.

for **10**, and 4.11 ppm ($J = 12.8$) for **9**, indicating that compounds **9** and **10** exist in the cone conformation [23].

Conclusions. – We have synthesized two new calix[4]arene derivatives which contain flavin units at the lower rim of calix[4]arene. The structures of flavin–calix[4]arene conjugates have been established by means of IR and ^{1}H-NMR spectroscopy, and elemental analyses.

It is well-known that the compounds which have N and O donor atom groups are able to inhibit the enzyme production [24]; therefore, **9** and **10**, which have several donor atoms, potentially could inhibit the enzyme production, and their biological

activity could be investigated *in vivo* and *in vitro*. In conclusion, **9** and **10** may be used for numerous applications in the medicinal area.

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Experimental Part

General. Solvents were dried by storing them over molecular sieves (*Aldrich*; 4 Å, 8–12 mesh). Toluene was dried with CaH_2 and stored over Na wire. DMF was dried with $CaSO_4$ and stored over molecular sieves. The drying agents employed during workup were Na_2SO_4 and $MgSO_4$. All aq. solns. were prepared with deionized H_2O that had been passed through a *Milli-Q Plus* water purification system. Starting materials and reagents were obtained from *Aldrich*, *Lancaster*, and *Fluka* and were used without further purification. TLC: *DC Alufolien Kieselgel 60 F_{254}* (*Merck*). M.p.: *Barnsted/Electro thermal* apparatus in a sealed capillaries; uncorrected. IR Spectra: *Perkin-Elmer 1605 FTIR System Spectrum BX* spectrometer; as KBr pellets. ^{1}H-NMR Spectra: *Varian 400* MHz and *Varian Mercury Plus 300* MHz spectrometers; chemical shifts in ppm rel. to TMS ($\delta = 0.0$ ppm). Elemental analyses (C, H, N): *Perkin-Elmer 240* analyzer.

*Syntheses. 5,11,17,23-Tetra(*tert*-butyl)-25,26,27,28-tetrahydroxycalix[4]arene* (**1**), *25,26,27,28-tetrahydroxycalix[4]arene* (**2**), *25,27-bis(3-cyanopropoxy)-26,28-dihydroxycalix[4]arene* (**3**), *25,27-bis(3-bromopropoxy)-5,11,17,23-tetra(*tert*-butyl)-26,28-dihydroxycalix[4]arene* (**4**), and *3-methylalloxazine* (**5**) were synthesized according to literature procedures [14][19][21][22]. *Ethyl (3-methylalloxazin-1-yl)acetate* (**6**), *(3-methylalloxazin-1-yl)acetic acid* (**7**), *(3-methylalloxazin-1-yl)acetyl chloride* (**8**), and the final products flavin–calix[4]arene **9** and flavin–calix[4]arene conjugates **9** and **10**, resp., were prepared for the first time according to the procedures described below.

25,27-Bis(3-cyanopropoxy)-26,28-dihydroxycalix[4]arene (=*4,4′-{[26,28-Dihydroxypentacyclo-[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diyl]bis-(oxy)}dibutanenitrile*; **3**). Prepared according to [21]. Yield 79%. M.p. 244–246°. IR (KBr): 2363 (CN). ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.39 (*quint.*, $J = 8.0$, 2 CH_2CH$_2$CN); 3.08 (*t*, $J = 7.2$, 2 CH$_2$CN); 3.47 (*d*, $J =$ 12.8, 2 ArCH_2Ar); 4.13 (*t*, $J = 5.6$, 3 OCH_2CH$_2$); 4.21 (*d*, $J = 12.8$, 2 ArCH_2Ar); 6.70 (*t*, $J = 7.2$, 2 arom. H); 6.79 (*t*, $J = 7.2$, 2 arom. H); 6.94 (*d*, $J = 7.2$, 4 arom. H); 7.09 (*d*, $J = 7.2$, 4 arom. H); 7.79 (*s*, 2 OH). Anal. calc. for $C_{36}H_{34}N_2O_4$ (558.68): C 77.40, H 6.13, N 5.01; found: C 77.01, H 6.22, N 4.92.

*25,27-Bis(3-bromopropoxy)-5,11,17,23-tetra(*tert*-butyl)-26,28-dihydroxycalix[4]arene* (=*26,28-Bis(3-bromopropoxy)-5,11,17,23-tetra-(*tert*-butyl)pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diol*; **4**). Prepared according to [22]. Yield 64%. M.p. 277–279°. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.05 (*s*, 4 arom. H); 4.32 (*d*, $J = 13.3$, ArCH_2Ar); 4.28 (*t*, $J = 5.6$, 2 CH$_2$O); 3.83 (*t*, $J = 6.4$, 2 CH$_2$Br); 3.32 (*d*, $J = 13.3$, 2 ArCH_2Ar); 1.29 (*s*, 2 *t*-Bu); 0.94 (*s*, 2 *t*-Bu). Anal. calc. for $C_{50}H_{66}Br_2O_4$ (890.88): C 67.41, H 7.47, Br 17.94; found: C 67.53, H 7.50, Br 17.93.

3-Methylalloxazine (= *3-Methylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione*; **5**). Prepared according to [14]. Yield: 65%. M.p. 285°. ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): 3.29 (*s*, MeN); 7.74–7.79 (*m*, 1 arom. H); 7.92 (*d*, $J = 3.5$, 2 arom. H); 8.18 (*d*, $J = 8.2$, 1 arom. H); 12.23 (*s*, NH). Anal. calc. for $C_{11}H_8N_4O_2$ (228.21): C 55.77, H 3.81, N 23.65; found: C 56.12, H 3.41, N 23.55.

Ethyl (3-Methylalloxazin-1-yl)acetate (= *Ethyl (3-Methyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-1(2*H*)-yl)acetate*; **6**). A mixture of **5** (0.3 g, 1.315 mmol), K_2CO_3 (1 g, 7.303 mmol), and $BrCH_2COOEt$ (2.195 g, 13.146 mmol) were stirred for 5 h in DMF (110 ml) at r.t. Then, the solvent was removed *in vacuo*, and the yellow product was crystallized from CH_2Cl_2/Et_2O. Yield: 0.318 g (77%). M.p. 205–207°. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz): 1.31 (*t*, $J = 6.9$, Me); 3.62 (*s*, MeN); 4.26 (*q*, $J = 7.5$, CH$_2$O); 5.20 (*s*, CH$_2$N); 7.77 (*t*, $J = 7.8$, 1 arom. H); 7.89 (*t*, $J = 6.8$, 1 arom. H); 7.99 (*d*, $J = 7.7$, 1 arom. H); 8.36 (*d*, $J = 8.2$, 1 arom. H). Anal. calc. for $C_{15}H_{14}N_4O_4$ (314.3): C 57.32, H 4.49, N 17.83; found: C 57.57, H 4.32, N 17.78.

(3-Methylalloxazin-1-yl)acetic Acid (=*(3-Methyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-1(2*H*)-yl)-acetic Acid*; **7**). A mixture of **6** (0.318 g, 1.012 mmol) and HCl (5 ml) was stirred at 80–90° for 105 min. The mixture was cooled, and ice-water (15 ml) was added to the soln. The yellow solid that precipitated was filtered off and washed with H_2O. Reprecipitation from 2N AcOH gave **7** as a light yellow solid.

Yield: 0.158 g (55%). M.p. 289–293°. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz): 3.31 (*s*, MeN); 4.98 (*s*, CH$_2$N); 7.85 (*t*, *J* = 6.3, 1 arom. H); 8.00 (*d*, *J* = 5.1, 2 arom. H); 8.26 (*d*, *J* = 7.8, 1 arom. H); 13.15 (*s*, OH). Anal. calc. for $C_{13}H_{10}N_4O_4$ (286.24): C 54.55, H 3.52, N 19.57; found: C 54.67, H 3.45, N 19.53.

(3-Methylalloxazin-1-yl)acetyl Chloride (= *(3-Methyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-1(2*H*)-yl)acetyl Chloride*; **8**). Compound **7** (1.00 g, 0.350 mmol) was added to $SOCl_2$ (2.5 ml, 31.903 mmol), and the suspension was stirred at 45°. After 2 h, the solid had dissolved, $SOCl_2$ was evaporated at < 45°, and the crude chloride **8** obtained was used without further purification.

*1,1′-{[5,11,17,23-Tetra-(*tert*-butyl)-26,28-dihydroxypentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diyl]bis(oxypropane-3,1-diyl)}bis(3-methylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione)*; **9**). K_2CO_3 (0.6 g), **5** (0.438 mmol), and NaI (0.4 g) were added to a soln. of **4** (0.219 mmol) in 20 ml of dry DMF, and the mixture was stirred under N_2 at r.t. for 47 h. The reaction was monitored by TLC. The salts were filtered off, and the solvent from the filtrate was removed under reduced pressure. Then, Et_2O was added, the precipitates formed were filtered off, and the received product was dried in a vacuum desiccator. Yield 92%. M.p. > 350°. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz): 8.53 (*s*, 2 OH); 8.07–7.93 (*m*, 4 arom. H); 7.76–7.76 (*m*, 2 arom. H); 7.58–7.48 (*m*, 2 arom. H); 7.10 (br. *s*, 8 arom. H); 4.62 (*t*, *J* = 6.4, 2 CH$_2$O); 4.11 (*d*, *J* = 12.8, 2 ArC*H*$_2$Ar); 4.0 (*t*, *J* = 5.2, 2 CH$_2$N); 3.35 (overlapped with DMSO, this area should correspond to 10 H-atoms belonging to ArC*H*$_2$Ar and MeN); 2.32–2.29 (*m*, 2 CH$_2$); 1.16 (*s*, 2 *t*-Bu); 1.10 (*s*, 2 *t*-Bu). Anal. calc. for $C_{72}H_{80}N_8O_8$ (1185.45); C 72.95, H 6.80, N 9.45; found: C 73.02, H 6.88, N 9.32.

*26,28-Bis(3-cyanopropoxy)pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3(28),4,6,9(27),10,12,15(26),16,18,21,23-dodecaene-25,27-diyl Bis[(3-methyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-1(2*H*)-yl)acetate]*; **10**). A mixture of **8** (0.11 g, 0.36 mmol), **3** (0.09 g, 0.164 mmol), and K_2CO_3 (0.10 g, 0.724 mmol) in MeCN (6 ml) was stirred at 85° for 37 h. After cooling, MeCN was removed to dryness, and the residue was dissolved in CH_2Cl_2, washed with H_2O (5 × 30 ml), dried (Na_2SO_4), and then concentrated to a third of the original volume on a rotary evaporation. The residue was then mixed with EtOH. Precipitated **10** was filtered off and dried under vacuum. Yield: 75 mg (42%). M.p. 340°. IR (KBr disk): 1740 (ester C=O). ^{1}H-NMR ((D$_6$)DMSO, 400 MHz): 2.32 (*quint.*, *J* = 6.1, 2 C*H*$_2$CH$_2$CN); 3.10 (*t*, *J* = 7.0, 2 CH$_2$CN); 3.36 (*s*, 2 MeN); 3.49 (*d*, *J* = 12.8, 2 ArC*H*$_2$Ar); 4.07 (*t*, *J* = 5.6, 2 CH$_2$O); 4.17 (*d*, *J* = 12.8, 2 ArC*H*$_2$Ar); 5.77 (*s*, 2 CH$_2$N); 6.63 (*t*, *J* = 7.5, 2 arom. H); 6.82 (*t*, *J* = 7.5, 2 arom. H); 7.08 (*d*, *J* = 7.6, 4 arom. H); 7.18 (*d*, *J* = 7.5, 4 arom. H); 7.80–7.83 (*m*, 2 arom. H); 8.01 (*d*, *J* = 2.3, 2 arom. H); 8.24 (*d*, *J* = 7.9, 4 arom. H). Anal. calc. for $C_{62}H_{50}N_{10}O_{10}$ (1095.14): C 68.00, H 4.60, N 12.79; found: C 67.97, H 4.51, N 12.93.

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