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CHEMICAL SYNTHESIS OF LAMPTEROFLAVIN AS LIGHT EMITTER IN THE LUMINOUS MUSHROOM, *Lampteromyces japonicus*

Hiroyuki Takahashi, Minoru Isobe* and Toshio Goto#

Laboratory of Organic Chemistry, School of Agriculture, Nagoya University

Chikusa, Nagoya 464-01, Japan

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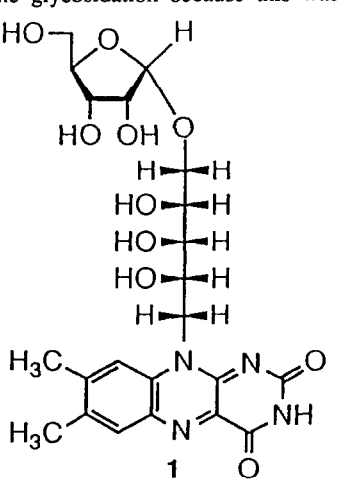
***Abstract** : The synthesis of lampteroflavin (the light emitter of the luminous mushroom, Lampteromyces japonicus) is described. Protected riboflavin was stereoselectively glycosylated with ribofuranosyl imidate to give largely the α-product Chloroethyl group which could be removed under neutral condition contributed to this synthesis.*

Lampteroflavin (Lf) **1** was isolated from the luminous mushroom, *Lampteromyces japonicus*, in 1987 by Isobe *et al* [1] as its bioluminescence light emitter. We are interested in the unique structure of **1** among natural product, that ribofuranoside is connected to 5'-oxygen of riboflavin with α-glycosidic bond. The isolated amount of **1** from nature was too small to study the bioluminescence mechanism of *L japonicus*, so we needed the synthesis of **1**. Concerned with the stability of lampteroflavin, the isoalloxazine moiety was decomposed under reductive or basic condition and the glycosidic bond was cleaved in aqueous acid. For these reasons, appropriate protective groups were selected for the chemical synthesis of lampteroflavin. Stereoselective synthesis of glycofuranosides, especially 1,2-cis-glycofuranoside, was known to be the most challenging problem.[2] We decided to employ imidate method[3] for the glycosidation because this was reported to be favorable for the SN2 type substitution. TBDPS (*tert*-butyldiphenylsilyl) and *p*-methoxybenzylidene groups were employed as the protective groups which could be deprotected under mild acidic or basic conditions

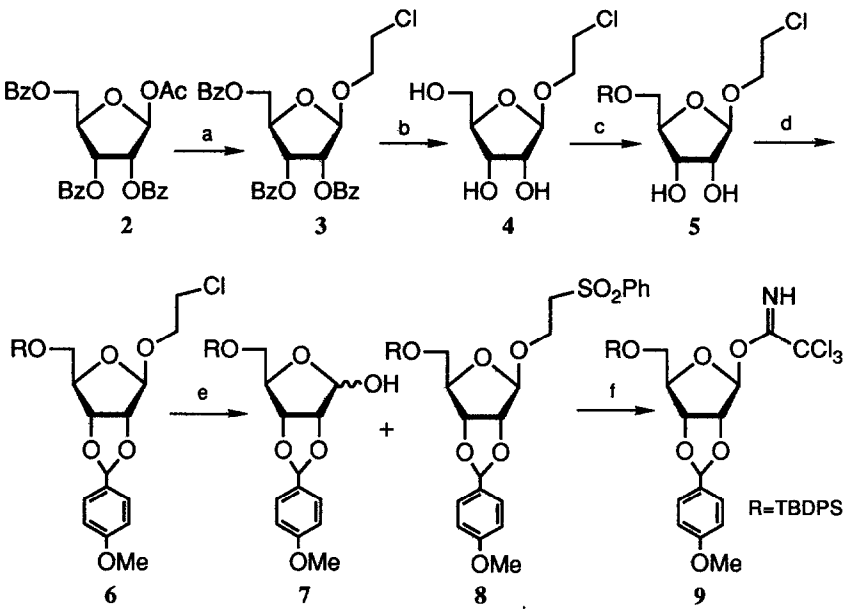
A commercially available starting material, 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose **2** was converted to 2-chloroethyl β-D-ribofuranoside **3** with *p*-TsOH (*p*-toluenesulfonic acid) in 2-chloroethanol at rt from the requirement of a later deglycosidation under non-acidic condition (**Scheme 1**) The benzoyl groups in **3** were hydrolysed with KOH in MeOH to give the triol **4** (in 98% 2-step overall yield), which was a mixture of α and β isomer in approximately 1 : 19 ratio determined from ^{1}H-NMR integration of 1-H (α : δ 5.18 ppm, d, J= 4.5 Hz, β : δ 5.06 ppm, s).[4] Selective protection of **4** with TBDPSCl (*tert*-butyldiphenylsilyl chloride) and imidazole in DMF



1

lampteroflavin

(N, N-dimethyl formamide) afforded the diol **5** in 93% yield, which was further converted to **6** with *p*-methoxybenzaldehyde dimethyl acetal, PPTS (pyridinium *p*-toluenesulfonate) in CH_2Cl_2. The excess *p*-methoxybenzaldehyde dimethyl acetal reagent was hydrolyzed to anisaldehyde in acetic acid, and this aldehyde was further reduced into more polar anisyl alcohol with $NaBH_4$ in EtOH. This alcohol was easily removed with column chromatography to afford **6** in 90% yield. The chloroethyl group in **6** was removed with sodium benzensulfinate and potassium iodide in DMF at 100°C to give **7** in 65% yield. The benzesulfinate existed as valence isomers between S (IV) and S (VI). In aprotic polar solvent, the sulfer atom in the later (VI) exhibited higher nucleophilisity toward chloromethyl carbon.[5] An intermediate **8** was isolated in 35% yield, which was also converted to **7** with KOH in EtOH in 50% yield. Treatment of **7** with trichloroacetonitrile in CH_2Cl_2 at 0°C gave trichloroacetimidate **9** as a thermodynamically stable β-isomer[6] in 65% yield.

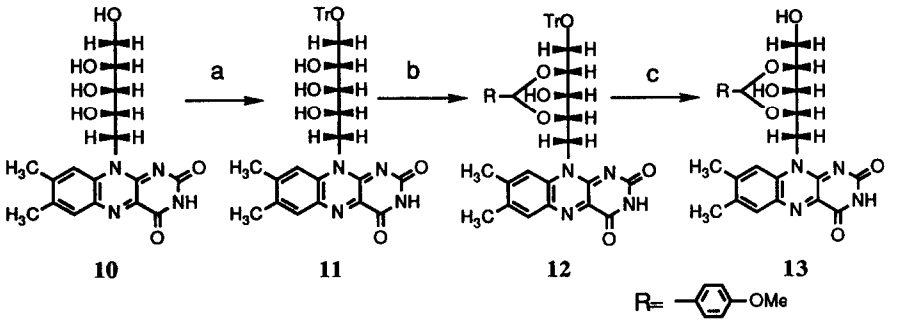


a) $HOCH_2CH_2Cl$ / *p*-TsOH rt 12 hr; b) 1% KOH / MeOH rt 1 hr 2-step overall yield 98%; c) TBDPSCl imidazole / DMF rt 30 min 93%; d) *p*-methoxybenzaldehyde dimethyl acetal / PPTS CH_2Cl_2 rt 1 hr AcOH-H_2O, $NaBH_4$ / EtOH 91%; e) $PhSO_2Na$ KI / DMF 100°C 6 hr 65%; f) CCl_3CN DBU / CH_2Cl_2 0°C 2 hr 65%.

Scheme 1

As a counterpart for the coupling with **9**, the partially protected **13** was synthesized from commercially available riboflavin **10** in 3 steps. Treatment of riboflavin with trityl chloride in pyridine at 110°C afforded **11** in 63% yield. It was quantitatively converted with *p*-methoxybenzaldehyde dimetyl acetal in the presence of PPTS in DMF into 2',4'-O-*p*-methoxybenzylidene acetal **12** in regioselective manner. The structure of **6** membered acetal was determined from the ^{1}H-NMR chemical shifts between **12** (3'-H, 3.5 ppm, m) and its

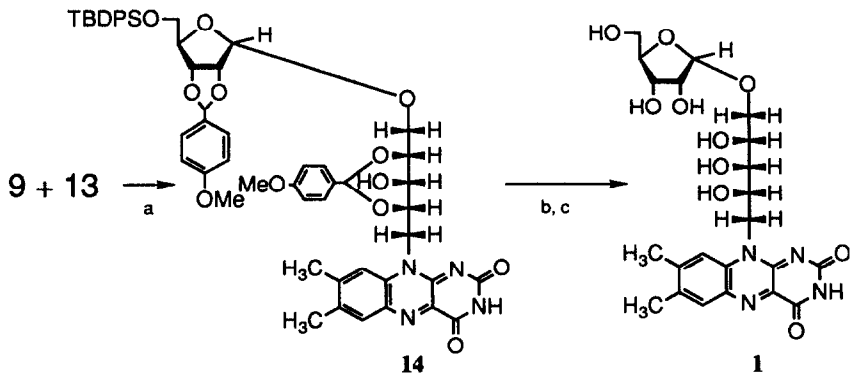
acetylated product (3'-H, 5.2 ppm, t, J= 9.2 Hz). Selective hydrolysis of the trityl group in the presence of *p*-methoxybenzylidene group was anticipated to be difficult because of their similar unstability under acidic conditions. We found that treatment of **12** with TFA in CH_2Cl_2 for 30 min gave **13** in relatively good yield (**Scheme 2**).



a) TrCl / Py 110°C 4 hr 63% ; b) *p*-methoxybenzaldehyde dimethyl acetal, PPTS / DMF rt quant; c) TFA / CH_2Cl_2 rt 30min 60%.

Scheme 2

The crucial coupling reaction between the imidate **9** and alcohol **13** with BF_3-Et_2O in CH_2Cl_2 at 0°C produced the glycoside **14** (**Scheme 3**). The ratio of the α-isomer [1-H; δ 5.09 ppm, d, J= 4.00 Hz: FAB-MS; m/z= 983 (M+1)] and β-isomer [1-H; δ 5.22 ppm, s: FAB-MS; m/z= 983 (M+1)] was estimated to be 4 : 1 after separation with TLC. The ratios of the α and β glycosidates were dependent upon the amount of BF_3-Et_2O.[7] The TBDMS group was cleaved with *n*-Bu_4NF in DMF and then the *p*-methoxybenzylidene group was removed with TFA in CH_2Cl_2 to yield **1**. In case of the α-glycoside, these deprotection reactions proceeded smoothly in good yield. The β-glycoside, however, the TBDPS group resisted under the same reaction condition as α-glycoside. A stronger condition caused considerable decomposition. This difference might be due to the hindered conformation of the β-glycoside.[8]



a) BF_3-Et_2O / CH_2Cl_2 0°C 6 hr 48% ; b) nBu_4NF / DMF rt 30min 80% ; c) TFA / CH_2Cl_2 rt 2 hr

Scheme 3

The physicochemical data of the synthesised lampteroflavin such as HPLC retention time and PMR (**Fig. 1**), FAB-MASS, UV and fluoresence spectra were identical with those of the natural lampteroflavin. The synthetic lampteroflavin has been provided for the aid of mechanistic studies on bioluminescence of *L. japonicus*. In other mechanistic studies on chemiluminescence with H_2O_2 in the presence of Fe(II), lampteroflavin exhibited the most efficient light production among other flavin compounds, such as riboflavin, lumiflavin, FMN, FAD, etc. The chemiluminescent studies will be reported shortly.

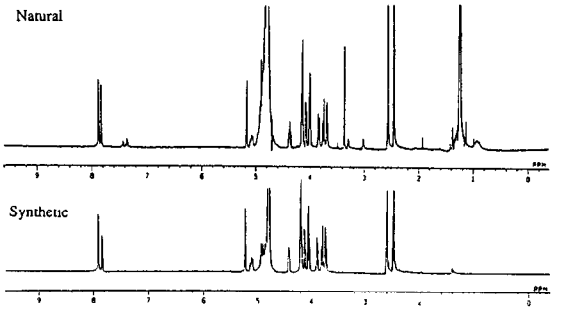


Fig. 1

500MHz ^{1}H-NMR spectrum identification of natural lampteroflavin (from *Lampteromyces japonicus*) and synthetic lampteroflavin

Experimental section

General Procedures. Proton and carbon nuclear magnetic resonance spectra were recorded on JEOL FX-90, FX-200, GSX-270, GX-500 spectrometers. All sample were dissolved in D_2O or $CDCl_3$ and chemical shifts are reported as δ value in parts per million relative to tetramethylsilane (^{1}H and ^{13}C 0 00ppm in $CDCl_3$) as internal standard. Coupling constants (J) are given in Hz. Mass spectra were recorded on JEOL DX-705L instrument. Fluorescence spectra were recorded on JASCO FP-770.

The reactions were carried out under a positive nitrogen atmosphere Reactions were monitored by thin layer chromatography (TLC) on Kieselgel silica 60 F254 plates. Column chromatography was carried out using Fuji Davison silica gel BW-820MH.

CH_2Cl_2 was distilled from CaH_2 for anhydrous reactions and DMF were dried with molecular sieves 4A.

2-Chroloethyl 2",3",5"-tri-O-benzoyl-β-D-ribofuranoside (3) A solution of the 1-O-acetyl-2, 3, 5-tri-O-benzoyl-β-D-ribofuranose **2** (89.0 g, 177 mmol) and *p*TsOH (18.3 g) in 2-chloroethanol (600 ml) was stirred overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and then poured into ice containing sat. $NaHCO_3$ solution. The aq layer was extracted with CH_2Cl_2 (×3), the combined organic layer was dried (Na_2SO_4) and concentrated under a reduced pressure to give the 2-chroloethyl 2",3",5"-tri-O-benzoyl-β-D-ribofuranoside **3** (122 g) as white crystals This material was used without further purification: ^{1}H-NMR (270MHz , $CDCl_3$) δ 3 57 (2H, t, J= 5 6), 3 75 (1H, dt, J= 6 7, 5 4), 4 01 (1H, dd, J= 5 6), 4 55 (1H, dd, J= 12 7, 6 6), 4 73-4 76 (2H), 5 31 (1H, s), 5.72 (1H, d, J= 5 0), 5 88 (1H, dd, J= 6 9, 5 0), 7 30-7 60 (9H), 7 89 (2H), 8.00-8 08 (4H) ppm. ^{13}C-NMR (22 5MHz, $CDCl_3$) δ 42.3, 64 9, 68 5, 72 4, 75 6, 79 4, 105 8, 130-132 ppm $[\alpha]_D$= +40 6° (c= 1 24, $CHCl_3$) FAB-MS m/z= 445 ($M-C_2H_4OCl$) Anal Calcd for $C_{28}H_{25}O_8$, C 64 12, H 4 77 Found C 64 19, H 4 85

2-Chloroethyl β-D-ribofuranoside (4). To a solution of the 2-chloroethyl 2",3",5"-tri-O-benzoyl-β-D-ribofuranoside **3** (112 g) in MeOH (400 ml) was added 3.4% KOH-MeOH (w/w, 150 ml) After stirring at rt for 3 hr, Dowex 50W-X about 10 g was added, the Dowex 50W-X was filtered with a glass filter and the

filtrate was concentrated under a reduced pressure. Column chromatography (silica gel 465 g, 0%-10% MeOH-CH_2Cl_2) of the residue afforded the 2-chloroethyl β-D-ribofuranoside **4** (35.6 g, 2-step overall yield 98.7%): ^{1}H-NMR (270MHz, D_2O) δ 3.66-3.90 (5H, m), 4.00-4.10 (2H, m), 4.14 (1H, d, J= 4.8), 4.24 (1H, dd, J= 7.2, 4.8), 5.10 (1H, s) ppm. ^{13}C-NMR (67.5MHz, D_2O) δ 43.3, 62.9, 68.1, 70.8, 74.2, 82.8, 106.9 ppm. [α]D= -45.8° (c= 0.73, MeOH). FAB-MS m/z= 133 ($M-C_2H_4OCl$).

2-Chloroethyl 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside (5). A solution of 2-chloroethyl β-D-ribofuranoside **4** (35.4 g, 166 mmol), *tert*-butyldiphenylsilyl chloride (60.3 ml, 1.25 eq.) and imidazole (25.0 g, 2.5 eq.) in DMF (530 ml) was stirred for 30 min at rt. The mixture was diluted with CH_2Cl_2 and then poured into ice containing sat. NH_4Cl solution. The aq. layer was extracted with CH_2Cl_2 (×3) and the combined organic layer was dried (Na_2SO_4) and concentrated under a reduced pressure. Column chromatography (silica gel 700 g, 2:3 ether-hexane) of the residue afforded the 2-chloroethyl 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside **5** as colorless syrup (69.8 g, yield 93.2%). ^{1}H-NMR (270MHz, $CDCl_3$) δ 1.07 (9H, s), 2.31 (1H, d, J= 5.2), 2.65 (1H, d, J= 3.4), 3.51 (2H, m), 3.63 (1H, m), 3.81 (3H, m), 4.05 (1H, dd, J= 5.7), 4.11 (1H, m), 4.34 (1H, dd, J= 5.0), 4.98 (1H, s), 7.40 (6H, m), 7.68 (4H, m) ppm. ^{13}C-NMR (50MHz, $CDCl_3$) δ 19.2, 26.8, 42.6, 65.1, 67.8, 72.0, 75.1, 83.5, 107.0, 127.5, 129.6, 133.0, 133.3, 135.3 ppm [α]D= -24.4° (c= 1.21, $CHCl_3$). Anal. Calcd for $C_{24}H_{31}O_5ClSi$; C 61.33, H 6.99. Found C 61.12, H 6.91

2-Chloroethyl 2",3"-O-*p*-methoxybenzylidene 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside (6). To a solution of 2-chloroethyl 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside **5** (66.6 g: 148 mmol) in CH_2Cl_2 (660 ml) were added *p*-methoxybenzaldehyde dimethyl acetal (48.4 ml, 2 eq.) and PPTS (19 g). After 1 hr, the reaction mixture was poured into ice containing sat. $NaHCO_3$ solution. The aq. layer was extracted with CH_2Cl_2 and the combined organic layer was dried (Na_2SO_4), concentrated under a reduced pressure to give a crude product (188.5 g). This was dissolved in a mixture solvent of AcOH (168 ml) and H_2O (6.7 ml) and stirred for 15 min. The reaction mixture was poured into ice containing sat. $NaHCO_3$ solution and neutralized with K_2CO_3. The aq. layer was extracted with CH_2Cl_2 (×3), dried ($NaSO_4$), and concentrated under a reduced pressure to give the crude residue (99.9 g). It was dissolved in EtOH (300 ml) and $NaBH_4$ (2 g) was added. The reaction mixture was stirred for 1hr, then AcOH (16.8 ml) was added and then concentrated under a reduced pressure. Column chromatography (silica gel 600 g, 1: 4 ether-hexane) of the resultant residue afforded the 2-chloroethyl 2",3"-O-*p*-methoxybenzylidene 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside **6** as colorless syrup (76.4 g, yield 90.8%): ^{1}H-NMR (270MHz, $CDCl_3$) δ 1.11 (9H, s), 3.45 (2H, m), 3.59 (1H, m), 3.71 (2H, d, J= 7.9Hz), 3.77 (1H, m), 3.81 (3H, s), 4.49 (1H, t, J= 7.9), 4.66 (1H, d, J= 6 4), 4.82 (1H, d, J=6,4), 5.19 (1H, s), 5 75 (1H, s), 6.91 (2H, d, J= 8.7), 7.41 (8H, m), 7.65 (4H, m) ppm ^{13}C-NMR (22.5MHz, $CDCl_3$) δ 19.4, 26.8, 42.4, 55.1, 64.3, 67 5, 81.4, 82.3, 84.1, 105 8, 107.9, 113.6, 127.6, 127 9, 128.2, 129 6, 135.3 ppm. [α]D= -29.0°(c=1.27, $CHCl_3$). FAB-MS m/z= 569 (M+1). Anal. Calcd for $C_{32}H_{37}O_6ClSi$, C 65.21, H 6 47. Found C 65.31, H 6 51

2",3"-O-*p*-Methoxybenzylidene 5"-O-*tert*-butyldiphemylsilyl-β-D-ribofuranoside (7). 2-Chloroethyl 2",3"-O-*p*-methoxybenzylidene 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside **6** (9.18 g, 16.2 mmol), KI (27.5 g), and sodium benzensulfinate (15.0 g; 5 eq) in DMF (100 ml) was stirred at 100°C overnight. The reaction mixture was diluted with ether and poured into water. The aq. layer was extracted with ether (×3) and the extracts were washed (H_2O, sat NaCl), dried (Na_2SO_4), and then concentrated under a reduced pressure. Column chromatography (silica gel 170 g, 1: 3 ether-hexane) of this residue afforded 2",3"-

O-*p*-methoxybenzylidene 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside **7** (5.54 g) as colorless syrup: ^{1}H-NMR (270MHz, $CDCl_3$) δ 1.10 (9H, s), 3.81 (3H, s), 3.6-3.9 (2H, m) , 4.46 (1H, m), 4.62 (1H, d, J= 10.5), 4.71 (1H, d, J= 5.9), 4.81 (1H, d, J= 5.9), 5.50 (1H, d, J= 10.5), 5.74 (1H, s), 6.8-7.0 (2H, m), 7.35-7.5 (12H, m), 7.6-7.8 (6H, m) ppm.

2",3"-O-*p*-Methoxybenzylidene 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl trichloroacetimidate (9). To a crude solution of 2",3"-O-*p*-methoxybenzylidene 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranoside **7** (4.37 g ; 8.64 mmol) in CH_2Cl_2 (21.7 ml), DBU (1.52 ml) and CCl_3CN (2.54 ml) were added dropwise at 0°C and stirred for 2hr. The reaction mixture was directly purified by column chromatography (silica gel 86 g, 1:1 ether-hexane) afforded 2",3"-O-*p*-methoxybenzylidene 5"-O-*tert*-butyldiphenylsilyl-β-D-ribofuranosyl trichloroacetimidate **9** (3.66 g, yield 65%) as colorless syrup: ^{1}H-NMR (270MHz, $CDCl_3$) δ 1.15 (9H, s), 3.65 (1H, t, J= 10.7), 3.77 (1H, dd, J= 5.0), 3.83 (3H, s), 4.66 (1H, dd, J= 10.7, 5.0), 4.84 (1H, d, J= 6.4), 4.95 (1H, d, J= 6.4), 5.80 (1H, s), 6.39 (1H, s), 6.93 (2H, d, J= 8.5), 7.3-7.5 (8H, m), 7.6-7.7 (4H, m), 8.46 (1H, brs) ppm.^{13}C-NMR (22.5MHz, $CDCl_3$) δ19.2, 26.8, 55.3, 63.7, 82.5, 85.1, 87.6, 105.8, 106.4, 113.9, 127.8, 128.1, 128.4, 129.1, 129.8, 132.9, 133.2, 135.5, 160.5, 161.0 ppm. $[\alpha]_D$= -42.7° (c= 0.88, $CHCl_3$). Anal. Calcd for $C_{31}H_{34}O_6NSiCl_3$; C 57.32, H 5.24, N 2.16. Found C 57.22, H 5.26, N 2.12.

5'-O-Trityl riboflavin (11). A solution of riboflavin **10** (3.00 g, 7.98 mmol) and TrCl (3.33 g, 1.5 eq) in pyridine (300 ml) was stirred at 110°C for 4hr, then cooled to rt. The reaction mixture was concentrated under a reduced pressure. Column chromatography (silica gel 70 g, 0%-3% MeOH-CH_2Cl_2) of the residue afforded the 5'-O-trityl riboflavin **11** (3.13 g, yield 63.4%) as yellow powder: ^{1}H-NMR (270MHz, $CDCl_3$) δ 2.43 (3H, s), 2.48 (3H, s), 3.43 (1H, dd, J= 8.6, 4.7), 3.53 (2H, m), 3.75 (1H, m), 4.02 (1H, m), 4.27 (1H, m), 4.60 (1H, d, J= 4.0), 4.72 (1H, d, J= 4.0), 4.83 (1H, dd, J= 12.7, 1.3), 5.12 (1H, dd, J= 12.7, 6.0), 7.2-7.3 (6H, m), 7.4-7.5 (9H, m), 7.88 (1H, s), 8.06 (1H, s), 8.74 (1H, brs) ppm.

2',4'-O-*p*-Methoxybenzylidene 5'-O-trityl-riboflavin (12). To a solution of 5'-O-trityl riboflavin **11** (7.98 g, 12.9 mmol) and *p*-methoxybenzaldehyde dimethyl acetal (24.0 ml) in DMF (300 ml), PPTS (645 mg) was added and the mixture was stirred at rt for 4hr. The reaction mixture was diluted with CH_2Cl_2 and poured into ice containing sat. $NaHCO_3$ solution. The aq. layer was extracted with CH_2Cl_2 (×3), dried ($NaSO_4$) and concentrated under a reduced pressure. Column chromatography (silica gel 210 g, 3%-5% MeOH-CH_2Cl_2) of the resultant residue afforded the 2',4'-O-*p*-methoxybenzylidene 5'-O-trityl-riboflavin **12** (9.50 g, quant.) as yellow powder: ^{1}H-NMR (270MHz, $CDCl_3$) δ 2.42 (3H,s), 2.43 (3H, s), 3.38 (1H, dd, J= 9.8, 5.7), 3.50 (1H, dd, J= 9.8, 2.8), 3.5 (1H, m), 3.80 (3H, s), 3.95 (1H, m), 4.26 (1H, m), 5.03 (2H, m), 5 60 (1H, s), 6.83 (2H, d, J= 8.8), 7.30 (2H, d, J= 8.8), 7.2-7 3, 7 4-7.5 (15H,m), 7.92 (1H,s), 8 03 (1H, s), 8 63 (1H,brs) ppm. ^{13}C-NMR (22.5MHz, $CDCl_3$) δ 19.4, 21.3, 47.6, 55 3, 64.0, 80.2, 86.5, 100.3, 113 4, 143 9 ppm $[\alpha]_D$= +56 6° (c= 0.40, $CHCl_3$), FAB-MS ; m/s= 737 (M+1), Anal. Calcd for $C_{44}H_{40}O_7N_4$, C 71.74, H 5.44, N 7 61. Found C 71 74, H 5 44, N 7.54

2',4'-O-*p*-Methoxybenzylidene riboflavin (13). To a solution of 2',4'-O-*p*-methoxybenzylidene 5'-O-trityl-riboflavin **12** (1.00 g, 1.36 mmol) in CH_2Cl_2 30ml, trifluoroacetic acid (0.6 ml) was added dropwise at rt. Stirred for 30 min, the reaction mixture was poured into ice containing sat. $NaHCO_3$ solution and the aq. layer was extracted (×3). The combined organic layer was dried (Na_2SO_4) and concentrated under a reduced pressure. Column chromatography (silica gel 40 g) of the residue with 5%MeOH / CH_2Cl_2 as eluent afforded 2',4'-O-*p*-methoxybenzylidene riboflavin **13** (297 mg, yield 43.9%) as yellow powder: ^{1}H-NMR

(270MHz, $CDCl_3$) δ 2.19 (1H, brs), 2.39 (3H, s), 2.44 (3H, s), 2.46 (1H, br), 3.8-4.0 (3H, m), 4.31 (1H, m), 4.94 (1H, brs), 5.32 (1H, brs), 5.63 (1H, s), 5.65 (1H, br), 6.81 (2H, dt, J= 8.5), 7.22 (2H, dt, J=8.5), 7.94 (1H, s), 8.06 (1H, s), 8.63 (1H, s) ppm. ^{13}C-NMR (67.5MHz, $CDCl_3$) δ 19.4, 21.4, 47 6, 55.3, 61.9, 77 1, 77.5, 79.1, 81.4, 100.5, 113.4, 117.9, 127.3, 129.5, 132.0, 132.5, 135.4, 137.7, 148.4, 150.3, 156.9, 159.8, 160.1 ppm. $[\alpha]_D$= -30.7° (c= 0.22, CH_2Cl_2+MeOH), FAB-MS m/z= 495 (M+1). Exact mass; Calcd for $C_{25}H_{26}O_7N_4$ 495.1872. Found 495.1864.

Coupling between 9 and 13. To a solution of 2',4'-O-*p*-methoxybenzylidene riboflavin **13** (132 mg, 0.268 mmol) in CH_2Cl_2 (25 ml), a solution of 2",3"-O-*p*-methoxybenzylidene 5"-O-*tert*-buthyldiphenylsilyl-β-D-ribofuranosyl trichloroacetimidate **9** (59.5 mg, 0.092 mmol) in CH_2Cl_2 (5ml) was added after cooling to 0°C, dil. BF_3-Et_2O (BF_3-Et_2O, 0.04 ml in Et_2O, 2 ml) 0.1 ml was added. After stirring overnight at 0°C, the reaction mixture was poured into ice containing sat. $NaHCO_3$. The aq. layer was extracted with CH_2Cl_2 and the combined organic layer was dried ($MgSO_4$) and concentrated under a reduced pressure. Purification of the residue on preparative silica gel tlc gave glycoside (α–product 14–α : 35.5 mg, β–product **14**-β : 10.0 mg; total yield 42%). **14**-α, ^{1}H-NMR (270MHz, $CDCl_3$) δ 1.07 (9H, s), 2.38 (3H, s), 2.52 (3H, s), 2.73 (3H, s), 3.76 (3H, s), 3 81 (1H, brs), 4.00 (3H, m), 4.31 (1H, brs), 4.39 (1H, brs), 4.58 (1H, m), 4.65 (1H, brs), 4.79 (1H, dd, J= 7.2, 2.3), 5.09 (1H, d, J= 4.0), 5.36 (1H, br), 5.62 (1H, s), 5.78 (1H, s), 5.82 (1H, br), 6.82 (2H, d, J= 8.6), 7.31 (2H, d, J= 8.6), 7.40-7.56 (8H, m), 7.65 (1H, s), 7.68-7.76 (4H, m), 7.82 (1H, s), 7.94 (1H, brs) ppm. ^{13}C-NMR (67.5MHz, $CDCl_3$) δ 19.3, 19.4, 21 4, 26.9, 45.6, 53 8, 55.3, 63.7, 77 2, 80.0, 80.1, 80.3, 81.2, 82 2, 99 3, 100.2, 108.9, 113.1, 113.2, 117.8, 127.2, 127.3, 127.7, 127 8, 129.34, 129.67, 129.8, 123.0, 131.9, 132.4, 133 2, 133.6, 134.7, 134.8, 135.7, 135.8, 136.7, 146 5, 150.4, 154.5, 159.3, 159.6, 159.7 ppm. $[\alpha]_D$= +159.4° (c= 0.42, $CHCl_3$), FAB-MS m/z= 983 (M+1) Exact mass: Calcd for $C_{54}H_{58}O_{12}N_4Si$ 983.3900; Found, 983.3901. **14**-β, ^{1}H-NMR (270MHz, $CDCl_3$) δ 0 88 (9H, s), 2 43 (6H, s), 3.74 (3H, s), 3.82 (3H, s), 3.75 (2H, m), 3.97 (3H, m), 4.10 (2H, m), 4.28 (1H, m), 4.40 (1H, m), 4.83 (1H, d, J=6.2), 4.91 (1H, dd, J=6.2, 2.5), 5.31 (1H, s), 5.41 (1H, brs), 5.52 (1H, s), 5 93 (1H, s), 6.72 (2H, d, J=8 7), 6.92 (2H, d, J=8.7), 7.06 (2H, d, J=8.7), 7.14-7.28 (6H, m), 7.42 (2H, d, J=8.7), 7.51 (4H, m), 7.57 (1H, s), 7.98 (1H, s), 8.47 (1H, s) ppm.

Deprotection of TBDPS group on 14-α. To a solution of **14**-α (36.4 mg, 0.037 mmol) in a mixture solution of CH_2Cl_2 (0.6 ml) and DMF (0.6 ml), *n*-Bu_4NF (0.08 ml) was added dropwise at room temperature with stirring for 30 min. The reaction mixture was extracted with CH_2Cl_2 (×3). The combined organic layer was concentrated under a reduced pressure. Purification of the residue on preparative silica gel TLC gave **15**-α(23.6 mg, yield 85.5%): ^{1}H-NMR (270MHz, $CDCl_3$) δ 2 37 (3H, s), 2 54 (3H, s), 2,65 (3H, s), 3,74 (3H, s), 3.67-3.81 (3H, m), 3 90-4 05 (3H, m), 4.34 (1H, d, J= 9.3), 4.41 (2H, m), 4.54 (1H, d, J= 9 3), 4.64 (1H, dd, J= 7.45, 3.7), 4 71 (1H, m), 4.78 (1H, dd, J= 7.5, 1.5), 5.03 (1H, d, J= 3 7), 5.42 (1H, brs), 5 54 (1H, brs), 5.63 (1H, s), 5 73 (2H, d, J= 8 7), 5.78 (1H, s), 6.80 (2H, d, J= 8.7), 7.27 (2H, d, J= 8 7), 7 51 (2H, d, J= 8 7), 7 62 (1H, s), 7.84 (1H, s)ppm. ^{13}C-NMR (67.5MHz, $CDCl_3$) δ 19.5, 21.5, 53.6, 55.3, 60.2, 61.9, 62 3, 79.3, 79 5, 79 9, 81.3, 83.3, 98.8, 100.2, 109.3, 113.1, 118.0, 127 1, 127.3, 129.3, 129.9, 131 8, 132.4, 134 8, 135.0, 136.9, 146.5, 150.2, 156.8, 159 5, 159 6, 159.7 ppm FAB-MS m/z= 494 (M+1) Exact mass; Calcd for $C_{38}H_{40}O_{12}N_4$ 745.2718 Found 745 2720.

Synthesis of lampteroflavin 1. To a solution of **15**-α (124 mg, 0.17 mmol) in CH_2Cl_2 (27.9 ml), TFA (0.2 ml) was added dropwise at rt and stirred for 2hr. The reaction mixture was poured into ice containing sat. $NaHCO_3$ solution and the organic layer was extracted with H_2O (×3). Direct column chromatography (Cosmosil 75 C_{18}-OPN, 0%-25% MeOH-H_2O) of aq. layer afforded the lampteroflavin. Purification with HPLC afforded **1** (52mg): ^{1}H-NMR (500MHz, D_2O) δ 2.49 (3H, s), 2.61 (3H, s), 3 73 (1H, dd, J= 12.3, 5.1), 3.81 (1H, dd,

J= 12.3, 3.4), 3.90 (1H, dd, J= 11.1, 2.6), 4.05 (2H, m), 4.12 (1H, dd, J= 6.0, 2.9), 4.20 (3H, m), 4.42 (1H, brs), 4.92 (1H, brs), 5.11 (1H, brs), 5.22 (1H, d, J= 4.0), 7.84 (1H, s), 7.91(1H, s) ppm. FAB-MS m/z= 509 (M+1), Anal. Calcd for $C_{22}H_{29}O_{10}N_4$; C 69.37, H 5.58, N 5.68. Found : C 69.37, H 5.58, N 5.73.

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7 The effect of the amount of Lewis acid (BF_3-Et_2O) was examined in model glycosidation reaction between 2,3,5-tri-O-benzyl-β-D-ribofuranosyl trichloroacetimidate and *n*-propanol. The concentration of catalyzed BF_3-Et_2O was extreamly critical: the ratio of α and β isomer varied from 2 : 1 (0.003 M BF_3-Et_2O) to 1 : 3.5 (0.18 M BF_3-Et_2O). Higher concentration of BF_3-Et_2O tended to larger amount of β-isomer through SN1 like mechanism. The use of large amount of BF_3-Et_2O in case of the glycosidation between **9** and **13**, however, lead to decompose of the product and starting material.

8 Lampteroflavin β-isomer was also synthesised in moderate yield. ^{1}H-NMR (500MHz, D_2O) δ 2.50 (3H, s), 2.62 (3H, s), 3.83 (2H, m, J= 11.8, 6.6), 3.92 (2H, m), 4.06 (1H, dd, J= 6.6, 3 1), 4.15 (2H, m, J= 3.1), 4.33 (1H, d, J= 4 4), 4.48 (1H, brs), 4.55 (1H, dd, J= 6.6, 4.4), 4.88 (1H, brs), 5.11 (1H, brs), 5.27 (1H, s), 7.86 (2H, s)ppm. FAB-MS m/z= 509 (M+1).