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FULL PAPER

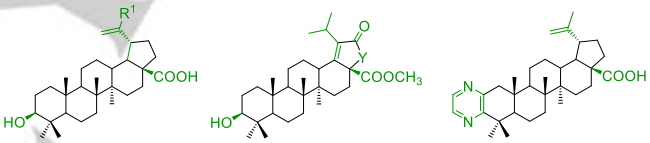
Synthetic approach for rapid preparation of BODIPY conjugates and their use in imaging of cellular drug uptake and distribution

Sona Krajcovicova,[b] Jarmila Stankova,[a] Petr Dzubak,[a] Marian Hajduch,[a] Miroslav Soural,* [b] and Milan Urban*[a]

Abstract: Solid-phase synthetic (SPS) method was developed for the preparation of BODIPY-labeled bioactive compounds that allows for fast and simple synthesis of conjugates usable in fluorescent microscopy. The approach was used to visualize cellular uptake and distribution of cytotoxic triterpenes in cancer cells.

Introduction

Visualization of small molecules in cells became an essential tool in drug discovery. The most commonly used method is fluorescent microscopy in which the studied molecule is equipped with a fluorescent label that allows direct visualization of the cellular uptake and distribution of the drug within the cell. A number of various conjugates of small molecules with a variety of fluorescent tags were reported to date with the application as probes,[1] photosensitizers[2] or luminescence switches and sensors.[3] Among them, BODIPY dyes[2,4] are preferably used fluorophores because of their superior physico-chemical properties such as high photostability, high quantum yield of fluorescence, total neutral charge and low polarity.[4] In contrast to other dyes, wavelengths of absorption and emission can be tuned easily by various substitutions on the BODIPY core.[4] The simplest BODIPYs show fluorescein-like parameters, however, unlike fluoresceins or rhodamines, they are prone to cellular permeability and lack nonspecific binding to proteins or lipids.[5] In this work, we designed a versatile solid-phase synthetic (SPS) method for the synthesis of fluorescent conjugates of biologically active molecules. The solid-phase synthesis allows for fast and simple production of libraries of desired compounds with only minimum effort and hands-on-time because it saves many isolation and purification steps of the intermediates. Surprisingly, there is only one example in the literature[6] that describes the use of SPS for adding substituents to the BODIPY core. However, there is no precedent of using the SPS in the synthesis of conjugates of BODIPY and other molecules. In agreement with this, there is a number of articles stating that BODIPY is incompatible with the SPS concept due to the lack of stability under the standard SPS reaction conditions.[3,6,7] Regardless of this statement, we have been able to develop a versatile procedure for the preparation of such conjugates using common backbone amide linker and standard coupling reagents by careful optimization of the reaction time and concentration of harsh reagents such as piperidine and trifluoroacetic acid. BODIPY-FL propanoic acid was selected as the most appropriate fluorescent label in this project. To prove the concept, we have synthesized a small set of BODIPY-labeled cytotoxic triterpenes in which we expected different mechanisms of action and differences in cellular uptake and distribution. Selected compounds (Figure **1**) have low micromolar cytotoxicity on various cancer cell lines, whereas some were supposed to have a unique mechanism of action: aldehyde **2**,[8] monoketone **3**,[9] diketone **4**,[9] and pyrazine **5**,[10] respectively (Figure **1**). Betulinic acid **1** was used as a standard since it is the most commonly studied cytotoxic triterpene and its mechanism of action has been well-studied to date.[11,12]



1; $R^1 = CH_3$ **3**; Y = CH_2 **5**
2; R^1 = CHO **4**; Y = CO

Figure 1. The selected triterpenoid structures.

Results and Discussion

Since the pharmacophores of the selected triterpenes are suggested but yet unproven, chemical modification at a wrong part of the molecule may negatively influence their biological behavior. Therefore, we decided to attach the fluorescent dye from three different sites and to compare the results. Triterpenes **1**-**5** were modified in positions C^3, C^{28} or C^{30} (Figure **2**).

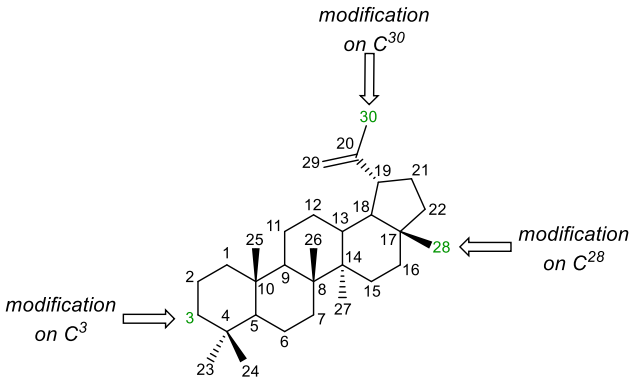


Figure 2. Three different sites to attach the fluorescent dye.

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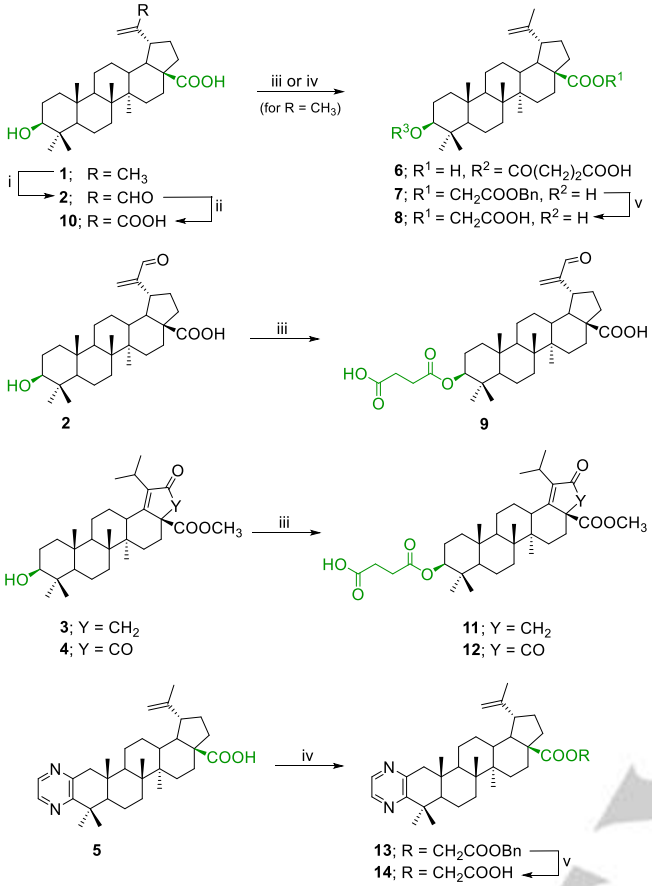
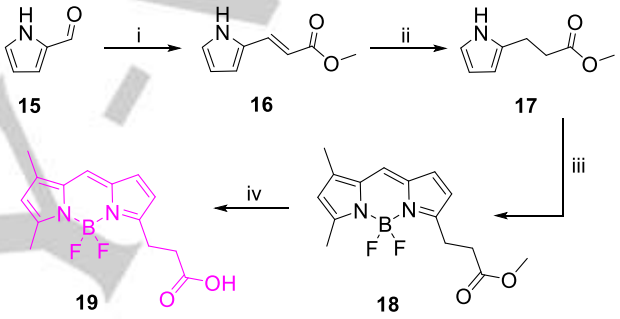


Scheme 1. Synthesis of premodified triterpenes. Reagents and conditions: i) SeO_2, diglyme, 140°C; ii) KH_2PO_4, $NaClO_2$, *t*-BuOH/2-methyl-2-butene (1:1), r.t. (**10**; 90%); iii) succinic anhydride, DMAP, THF/DMF (2:1), 80°C (**6**; 80%, **9**; 73%, **11**; 73%); iv) benzylbromoacetate, K_2CO_3, THF, 50°C (**7**; 98%, **13**; 91%); v) Pd/C, H_2, CH_3OH, r.t. (**8**; 84%, **14**; 49%).

Because of the steric hindrances at some of the selected positions, the original triterpenes had to be premodified by hemisuccinate at C^3-OH, glycolate at C^{28}OOH, or oxidized to carboxylate at C^{30} (Scheme **1**, see the experimental part [EP] and the SI file). Briefly, the C^3 modified hemisuccinates **6**, **11** and **12** were obtained after the reaction of betulinic acid **1**, monoketone **3** or diketone **4**, with succinic anhydride in the presence of a base. C^3 modified hemisuccinic aldehyde **9** was obtained after two-stage oxidation of betulinic acid **1** with selenium dioxide, followed by abovementioned reaction of aldehyde **2** with succinic anhydride. C^{28} modification of compounds **1** and **5** yielded protected glyoxalates **7** and **13**, which upon catalytic hydrogenation in the presence of Pd/C afforded desired modified triterpenes **8** and **14**, respectively. Finally, the compound **10** was achieved after facile and chemoselective Pinnick oxidation of the aldehyde **2**, which formed the carboxyl at the C^{30} position.

Synthesis of BODIPY-FL propanoic acid had to be optimized (Scheme **2**, see the EP and the SI file) to obtain better yields than those previously reported in the literature.[13,14] Although BODIPY-FL propanoic acid **19** (Scheme 2) is commercially available, due to its extremely high price we synthesized the dye by ourselves.

The reported syntheses[13,14] had to be slightly modified and optimized to increase the overall yield. It started from the commercially available pyrrole-2-carbaldehyde **15**, which was firstly converted to α,β-unsaturated ester **16** *via* the Horner–Wadsworth–Emmons reaction with excellent selectivity yielding only (*E*)-alkene. Such selectivity was obtained due to the formation of stabilized ylide. The following reduction provided intermediate **17**, which was then subjected to $POCl_3$ promoted coupling with commercially available 3,5-dimethyl-1H-pyrrole-2-carbaldehyde. Final treatment with $BF_3.OEt_2$ yielded BODIPY-FL propanoate **18** in one-pot reaction sequence (Scheme **2** in main manuscript). Subsequent acidic hydrolysis of ester yielded final BODIPY-FL propanoic acid **19** in excellent overal yield of 40 % (Scheme **2**), indicating unusually long kinetic stability of BODIPY dye in acidic media.[15] Importantly, the improved synthesis of BODIPY-FL propanoic acid is scalable up to gram-scale quantities, which is the solution to the most common problem in the synthesis of BODIPY dyes.



Scheme 2. The synthetic route for the preparation of BODIPY-FL propanoic acid **19**. Reagents and conditions: i) methyl(triphenylphosphoranylidene)acetate, CH_2Cl_2, r.t., 84%; ii) Pd/C, H_2, CH_3OH, r.t., 90%; iii) a) 3,5-dimethylpyrrole-2-carbaldehyde, $POCl_3$, CH_2Cl_2, 0°C to r.t., b) $BF_3.OEt_2$, DIPEA, CH_2Cl_2, 0°C to r.t., 63%; iv) THF/H_2O/conc. HCl (3:2:1), 0°C to r.t., 85%.

In contrast to the synthesis of biotin-preloaded resins,[16,17] the procedure for the preparation of BODIPY-preloaded resin **23** had to be carefully optimized due to limited chemical stability of BODIPY-FL. The aminomethyl resin was equipped with backbone amide linker (BAL) and subjected to reductive amination with 2-(2-aminoethoxy)ethanol to obtain immobilized secondary amine **20** (Scheme **3**). The chemoselective protection of the secondary amine with Fmoc was followed by acylation of the hydroxy group with prepared BODIPY-FL propanoic acid **19** and afforded resin **21**. Cleavage of the Fmoc-protective group and acylation with [2-[2-(Fmoc-amino)ethoxy]ethoxy]acetic (FAEEAA) acid using the standard DIC/HOBt technique yielded Fmoc-protected resin **22** which, upon deprotection with low concentration of piperidine in DMF, yielded desired preloaded resin **23** in very good crude purity (82%; calculated from UHPLC-MS traces). Subsequent acylation with the premodified triterpenes **6, 8-12** and **14** afforded the final conjugates **24-28, 30** and **31** (Scheme **3**).

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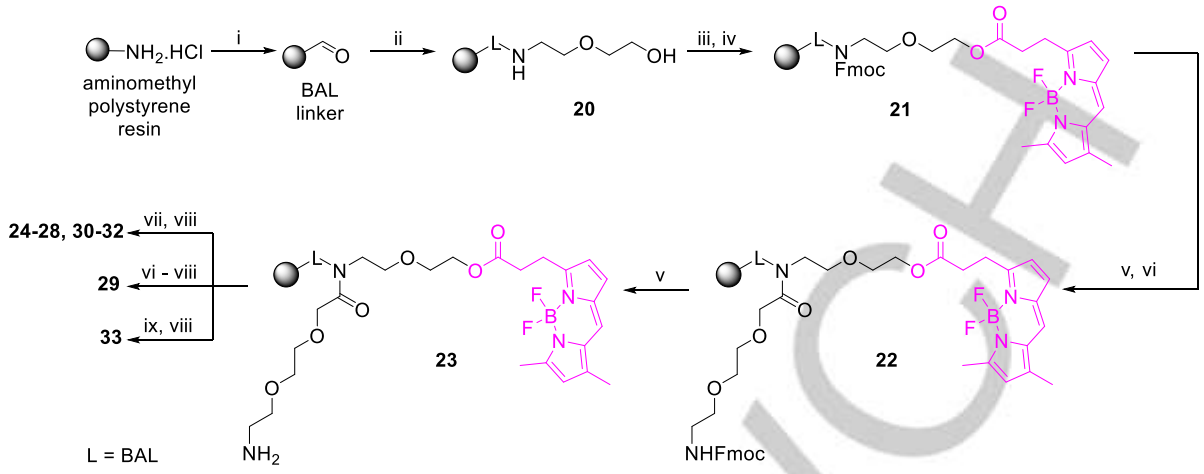

Scheme 3. Synthesis of BODIPY-preloaded resin and its use for conjugation with triterpenes. Reagents and conditions: i) BAL, HOBt, DIC, DMF/CH_2Cl_2 (1:1), r.t.; ii) 2-(2-aminoethoxy)ethanol, $NaBH(OAc)_3$, DMF/AcOH (10:1), r.t.; iii) FmocOsu, CH_2Cl_2, r.t.; iv) **19**, HOBt, DIC, DMF/CH_2Cl_2 (1:1); v) DMF/piperidine (20:1), r.t.; vi) FAEEAA, DIC, HOBt, DMF/CH_2Cl_2 (1:1), r.t.; vii) **6, 8-12, 14**, DIC, DMF/CH_2Cl_2 (1:1), r.t.; viii) CH_2Cl_2/TFA (10:1), r.t.; ix) Ac_2O, DMAP, CH_2Cl_2, r.t.

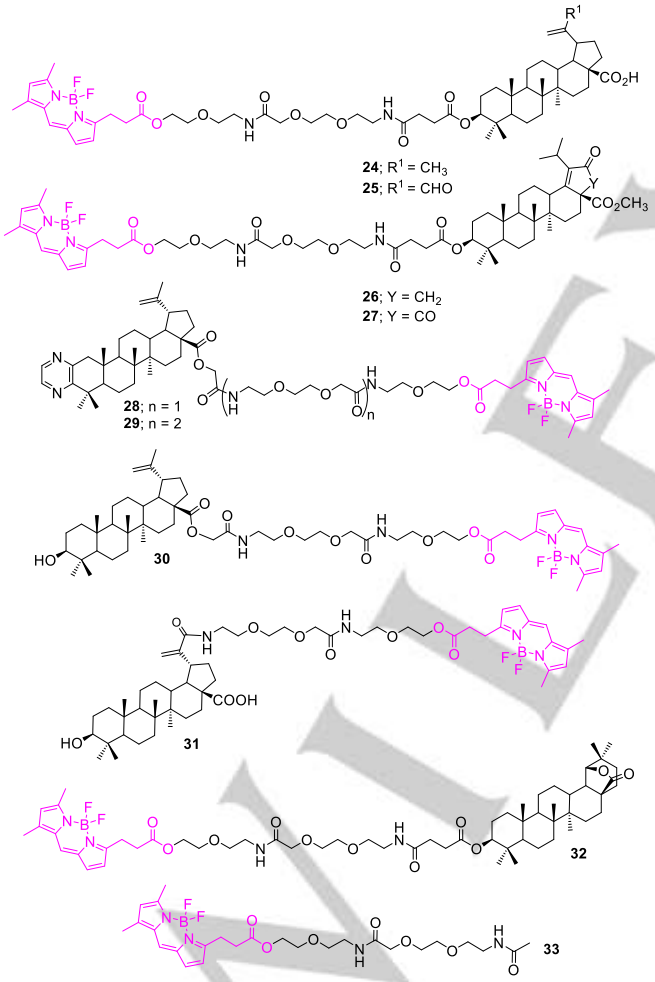

Figure 3. Structures of prepared conjugates **24** – **33**. Compounds **32** formed as a byproduct and was not further studied since it lacks the cytotoxic activity.

Compound **29** containing an extended linker was prepared to investigate the influence of the length of the linker on the cellular uptake or distribution and compound **33** was designed to show the properties of the BODIPY-FL connected to linker without a triterpene. In contrast to the construction of the intermediate **23**, omitting HOBt in the final acylation with triterpenes (Scheme **3**, step vii) increased the final crude purities of all conjugates significantly. Additionally, TFA-mediated cleavage of the conjugate **24** from the resin led to the formation of byproduct **32** in the equimolar ratio (calculated from UHPLC-MS traces) as a result of Wagner-Meerwein rearrangement.[18] It is important to mention, that the concentration of both piperidine and TFA had to be considerably lowered, compared to standard cleavage conditions (20-50% piperidine in DMF; 25-50% TFA in CH_2Cl_2), to maintain good crude purities of all intermediates as well as final conjugates and to prevent decomposition of BODIPY scaffold (see SI for the details).

The excitation and emission spectra of BODIPY-FL propanoic acid **19** were measured and compared to BODIPY conjugates **24-33**. All of the conjugates **24-33** had the same absorption/emission wavelengths and Stokes shifts as the parent BODIPY-FL propanoic acid (See SI for details). The conjugate's quantum yields of fluorescence are lower in comparison to BODIPY-FL propanoic acid (Φ = 98 for **19** *vs.* Φ = 0.14 - 0.30 for compounds **24-32**). The diminished fluorescence is probably caused by the static quenching between BODIPY dye and triterpenes, which is consistent with recently published data.[19,20]

The cytotoxic activity of the parent compounds and fluorescent conjugates was investigated *in vitro* against eight human cancer cell lines and two non-tumor fibroblasts by using the standard MTS test (Table **1**). The cancer cell lines were derived from T-lymphoblastic leukemia CCRF-CEM, leukemia K562 and their multiresistant counterparts (CEM-DNR, K562-TAX), solid tumors including lung (A549) and colon (HCT116, HCT116p53-/-) carcinomas, osteosarcoma cell line (U2OS), and for comparison, on two human non-cancer fibroblast lines (BJ, MRC-5). In general, the CCRF-CEM cell line was the most sensitive cancer cell line to the prepared compounds with only a few exceptions. Therefore, SARs assumptions were mostly based on the activities in CCRF-CEM cells.

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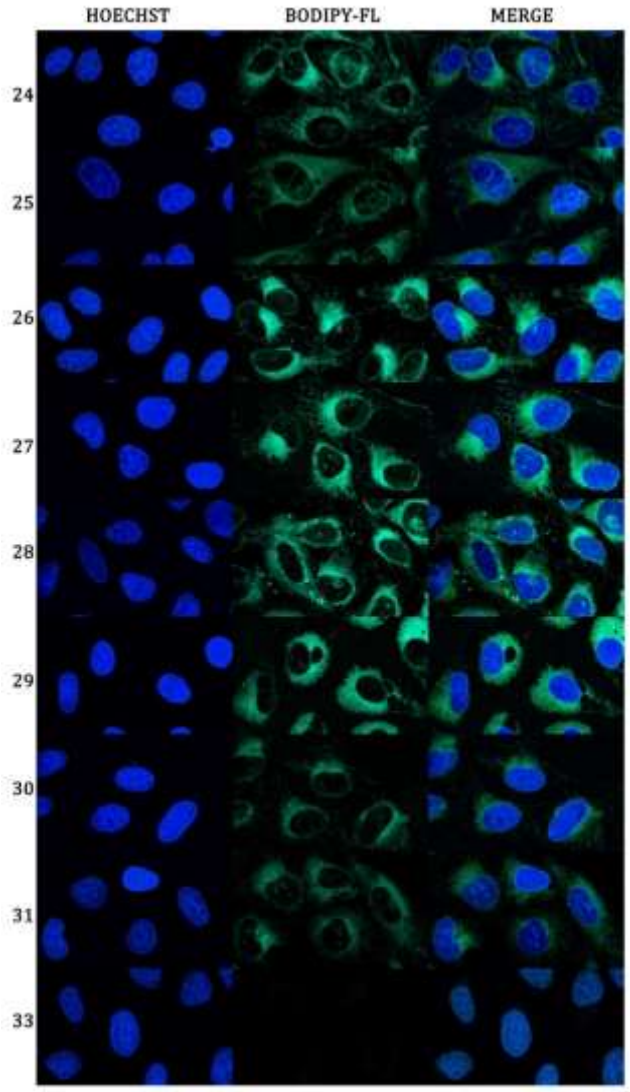

Figure 4. Fluorescence imaging of U2OS cells stained by BODIPY-triterpene conjugates. (For full resolution see SI).

Among the unmodified studied molecules **1**-**5**, aldehyde **2** and pyrazine **5** were cytotoxic against the CCRF-CEM line in a low micromolar range of 1.53 and 0.53 μM, respectively. The therapeutic index is rather low for the aldehyde **2** (4.7) but surprisingly high in the case of pyrazine (more than 94). The synthesized fluorescent conjugate of aldehyde **25** remained highly but unselectively cytotoxic, probably due to the presence of the reactive acrolein moiety. On the other hand, fluorescent conjugates of pyrazine (**28** and **29**) had slightly decreased activity and selectivity in comparison with the parent compound **5**, which indicates an important role of the free carboxyl group as a pharmacophore. Interestingly, the length of the linker is affecting the activity of the conjugates as well. This may be indicated by the comparison of conujgates **28** and **29** from which the longer one (**29**) was more active than the shorter one (**28**). Lastly, the conjugate of diketone **27** was cytotoxic only on CCRF-CEM cell line, whereas the monoketone conjugate **26** completely lost its cytotoxic activity and selectivity. Conjugate of betulinic acid **24** at the position C^3 remained active, nevertheless, its conjugates at the positions C^{28} and C^{30} (compounds **30** and **31**,) were almost inactive.

In fluorescent microscopy experiments (which were performed in early intervals before the cytotoxic effect took place), we have observed that all the tested fluorescent conjugates of triterpenes are staining living cells and passing through the cellular membrane into the cytoplasmic compartment (Figure 4, full resolution image is in the SI). In addition, we used the BODIPY conjugate **33** (which has the active triterpenic scaffold replaced by acetate) as a negative control. According to the results, it is not penetrating the cellular membrane indicating that it is the triterpenoid part which is responsible for the cellular uptake. This is likely because of the high lipophilicity of triterpenes. The conjugate **25** containing Michael acceptor (acrolein moiety in this case) resulted in different staining pattern - labeling cellular cytoplasm homogenously, which is presumably caused by non-specific covalent interaction with multiple intracellular proteins. Staining is distinctly apparent when compared to another tested compounds (**24**, **26**, **27**, **28**, **29**, **30**, **31**) which were labeling more subtle cytoplasmic and membrane structures, likely mitochondria, endoplasmic reticulum (ER), and the nuclear membrane. Co-staining experiments are currently being performed to confirm this unambiguously. Such results are in agreement with precedent studies of another lupane triterpenes that were found to interact with mitochondrion and ER.[21,22]

Conclusions

In this work we optimized the synthesis of BODIPY-FL propanoic acid **19** to give better yields than procedures reported by other authors and the synthesis is suitable for multi-gram scale quantities. We prepared BODIPY-preloaded resin and applied it to attach the fluorescent dye to cytotoxic triterpenic derivatives. Despite previous reports on the limited applicability of BODIPYs in solid-phase synthesis, due to their low stability under both basic and acidic conditions,[3,6,7] we managed to develop and optimize the synthetic protocols to overcome these problems. The reported preloaded resin allows for routine and simple connecting of various compounds to BODIPY label through a linker of choice using simple laboratory equipment, common coupling reagents and conditions, minimum hands-on-time and it can be even commercialized similarly to biotin-preloaded resin (Biotin NovaTag™, Novabiochem). Nine conjugates of BODIPY with cytotoxic triterpenes were synthesized using the resin **23** and their spectroscopic and biological properties were evaluated. In order to prove that BODIPY with the linker do not interfere with the biological study, we prepared a conjugate in which the triterpenic part was replaced with acetate. Live cell studies focused on fluorescence conjugates uptake have demonstrated non-specific labeling in the aldehyde **25** and more specific labeling pattern in the case of conjugates **24**, and **26**-**31**. Ongoing research is now focused on more specific determination of which organelles, proteins or protein complexes are targeted by our conjugates, and

it will be the aim of further proteomic and molecular biology studies, e.g. co-localization experiments.

Table 1. Cytotoxic activity of all prepared compounds.

	IC_{50} (μM/L)[a]										
Comp.	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53-/-	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
1[c]	8.09	14.04	4.29	14.09	9.43	15.78	15.96	20.75	24.23	28.18	3.24
2[c]	1.53	7.66	8.83	12.43	9.68	7.73	7.23	7.51	11.99	2.39	4.70
3[c]	15.16	20.16	27.25	34.67	21.01	24.25	27.12	40.06	44.28	42.67	2.87
4[c]	35.58	35.98	>50	43.11	>50	>50	>50	>50	>50	>50	> 1.41
5[c]	0.53	0.63	11.54	11.6	31.84	34.41	47.3	32.43	>50	>50	> 94.34
24	6.62	>50	>50	>50	>50	>50	>50	33.75	43.61	43.6	6.59
25	0.76	6.06	1.65	10.52	1.96	1.6	1.45	1.46	1.87	1.62	2.30
26	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	N.A.
27	3.4	>50	45.7	>50	>50	>50	>50	>50	45.84	44.26	13.25
28	18.09	20.28	>50	>50	>50	>50	>50	>50	>50	>50	> 2.76
29	6.13	9.27	18.92	12.07	29.02	35.05	29.14	26.34	39.74	38.23	6.36
30	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	N.A.
31	41.17	40.68	41.96	>50	49.02	40.6	41.43	45.02	48.81	40.75	1.09
32	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	N.A.
33	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	N.A.

[a]The lowest concentration that kills 50 % of the cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value.
[b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs. an average of both fibroblasts (BJ and MRC-5).
[c]Parent compounds used as a standard. Compounds with IC_{50} > 50 μM are considered inactive.

Experimental Section

General technical information are available in the Supporting information file and are analogous to our previous publications.[23,24] Note that the yields of the final conjugates (**24**-**33**, usually between 10-30 %) are calculated as overall yields of the entire synthetic procedure between compound **20** and the final product in the **scheme 3**.

General procedure for the preparation of benzyl glyoxalates. To a stirred solution of starting material in THF were added benzyl bromoacetate (3 eq) and K_2CO_3 (3 eq). The reaction mixture was stirred at 50 °C and monitored by TLC (hexane/EtOAc = 3:1, v/v) which indicated its completion after overnight stirring. The reaction mixture was concentrated, diluted with water (100 mL/ 1.32 mmol) and extracted with EtOAc (5x 100 mL/ 1.32 mmol). Organic extracts were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude products were purified by flash chromatography (hexane/EtOAc = 3:1 to 1:1, v/v) to yield desired compounds.

2-(Benzyloxy)-2-oxoethyl betulinate (7). Compound **7** was prepared by the General Procedure A using **1** (600 mg, 1.32 mmol), benzyl bromoacetate (633 μL, 4 mmol) and K_2CO_3 (552 mg, 4 mmol) in THF (20 mL); compound **7** was obtained as a white solid (783 mg, 98% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 – 7.35 (m, 5H, Ph), 5.22 (d, *J* = 6.5 Hz, 1H, O**CH$_2$**Ph), 5.18 (d, *J* = 12.3 Hz, 1H, O**CH$_2$**Ph), 4.73 (d, *J* = 2.0 Hz, 1H, H$^{29\ pro\text{-}Z}$), 4.65 (d, *J* = 2.3 Hz, 2H, O**CH$_2$**CO), 4.61 – 4.60 (m, 1H, H$^{29\ pro\text{-}E}$), 3.21 – 3.17 (dd, *J* = 11.0 Hz, 4.8 Hz, 1H, H$^{3\alpha}$), 2.97 (td, *J* = 11.3, 10.9 , 4.7 Hz, 1H, H$^{19\beta}$), 2.30 (dt, *J* = 12.6, 2.7 Hz, 1H), 2.27 (td, *J* = 13.5, 13.4, 3.6 Hz 1H), 2.06 – 1.99 (m, 1H), 1.96 – 1.86 (m, 1H), 1.72 – 1.13 (m, 24H, *overlap with solvent*), 0.97 (s, 6H, 2x CH_3), 0.92 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.77 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 175.6, 168.1, 150.7, 135.4, 128.8, 128.6, 109.8, 79.2, 67.2, 60.4, 56.7, 55.6, 50.8, 49.6, 47.0, 42.6, 40.9, 39.0, 38.9, 38.3, 37.4, 37.1, 34.5, 32.1, 30.6, 29.8, 28.2, 27.6, 25.8, 21.1, 19.6, 18.5, 16.3, 16.1, 15.5, 14.9 ppm. HRMS (ESI): *m/z* calcd for $C_{39}H_{56}O_5$ $[M+H]^+$ = 605.4201, found $[M+H]^+$ = 605.4207.

Benzyl glyoxalate of betulinic acid pyrazine (13). Compound **13** was prepared by the General Procedure A using **5** (100 mg, 0.2 mmol), benzyl bromoacetate (96 μL, 0.61 mmol) and K_2CO_3 (84 mg, 0.61 mmol) in THF (8 mL); compound **13** was obtained as a yellowish oil (116 mg, 91% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (d, *J* = 2.1 Hz, 1H, pyrazine), 8.17 (d, *J* = 2.4 Hz, 1H, pyrazine), 7.33 – 7.24 (m, 5H, Ph, *overlap with solvent*), 5.14 – 5.06 (m, 2H, O**CH$_2$**Ph), 4.66 – 4.54 (m, 4H), 2.96 – 2.86 (m, 3H), 2.37 – 2.21 (m, 4H), 1.96 – 1.91 (m, 1H), 1.86 – 1.80 (m, 1H), 1.70 – 1.22 (m, 17H, *overlap with solvent*), 1.20 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 0.92 – 0.90 (m, 6H, 2x CH_3), 0.70 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 175.5, 168.1, 159.8, 151.0, 150.5, 142.4, 141.6, 135.3, 128.8, 128.7, 128.6, 109.9, 67.2, 60.4, 56.7, 53.2, 49.5, 49.0, 48.9, 46.9, 42.6, 40.7, 39.6, 38.3, 37.1, 36.9, 33.4, 32.0, 31.7, 30.6, 29.7, 25.7, 24.2, 21.6, 20.2, 19.6,

16.3, 15.7, 14.8 ppm. HRMS (ESI): *m/z* calcd for $C_{41}H_{54}N_2O_4$ $[M+H]^+$ = 639.4156, found $[M+H]^+$ = 639.4155.

3β-Hydroxylup-20(29)-ene-28,30-dioic acid (10). To a stirred solution of aldehyde **2** (100 mg, 0.21 mmol) in *t*-BuOH/2-methyl-2-butene (1:1, 10 mL, v/v) was added $NaClO_2$ (96 mg, 1.06 mmol) and solution of KH_2PO_4 (550 mg, 4.04 mmol) in H_2O (5 mL). The reaction mixture was stirred vigorously at ambient temperature and monitored by TLC (CH_2Cl_2/CH_3OH = 10:1, v/v), which indicated its completion after 5 h. The reaction mixture was concentrated, diluted with NH_4Cl (50 mL) and extracted with EtOAc (5x 50 mL). Organic extracts were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/CH_3OH = 5:1, v/v) to afford compound **10** as a white crystalline solid (90 mg, 90% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.17 (br s, 2H, 2x COOH), 5.96 (s, 1H, $H^{29\ pro\text{-}Z}$), 5.60 (s, 1H, $H^{29\ pro\text{-}E}$), 4.25 (d, *J* = 5.1 Hz, 1H), 4.08 – 4.07 (m, 1H), 2.98 – 2.94 (m, 1H), 2.22 – 2.07 (m, 3H), 1.99 – 1.91 (m, 1H), 1.82 – 1.75 (m, 2H), 1.62 – 1.52 (m, 2H), 1.43 – 1.24 (m, 16H), 0.90 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.76 (s, 3H, CH_3), 0.65 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6): δ 177.2, 168.4, 147.7, 122.5, 76.7, 55.5, 54.8, 51.05, 49.8, 48.6, 41.9, 38.5, 38.2, 37.3, 36.7, 36.0, 33.9, 32.7, 31.6, 29.2, 28.1, 27.1, 26.8, 20.5, 17.9, 15.9, 15.8, 15.7, 14.3 ppm. HRMS (ESI): *m/z* calcd for $C_{30}H_{46}O_5$ $[M-H]^-$ = 485.3262, found $[M-H]^-$ = 485.3252.

General procedure B for preparation of hemisuccinates. To a stirred solution of starting material in THF/DMF (2:1) was added succinic anhydride (6 eq) and DMAP (6 eq). The reaction mixture was stirred at 80 °C and monitored by TLC (CH_2Cl_2/CH_3OH = 10:1, v/v) which indicated its completion after 36 to 48 h. The reaction mixture was concentrated, diluted with NH_4Cl (150 mL/ 2.2 mmol) and extracted with EtOAc (5x 100 mL/ 2.2 mmol). Organic extracts were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude products were purified by flash chromatography (CH_2Cl_2/CH_3OH = 10:1, v/v) to afford desired compounds.

Betulinic acid 3-hemisuccinate (6). Compound **6** was prepared by the General Procedure B using **1** (1 g, 2.2 mmol), succinic anhydride (1.32 g, 13.1 mmol) and DMAP (1.6 g, 13.1 mmol) in THF/DMF (2:1, 60 mL) for 36 h; yield compound **6** as a white solid (980 mg, 80% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.14 (br s, 2H, 2x COOH), 4.69 (s, 1H, $H^{29\ pro\text{-}Z}$), 4.56 (s, 1H, $H^{29\ pro\text{-}E}$), 4.37 (dd, *J* = 11.7, 4.6 Hz, 1H, $H^{3\alpha}$), 2.97 – 2.92 (m, 1H, $H^{19\beta}$), 2.54 – 2.46 (m, 4H, *overlap with solvent*) 2.25 – 2.18 (m, 1H), 2.11 (m, 1H), 1.79 (m, 1H), 1.64 (s, 3H, CH_3), 1.62 – 1.07 (m, 12H), 0.94 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.78 (s, 6H, 2x CH_3). *All other data were consistent with published results.*[6]

30-Aldehyde 3-hemisuccinate of betulinic acid (9). Compound **9** was prepared by the General Procedure B using **2** (1.5 g, 3.13 mmol), succinic anhydride (1.88 g, 18.78 mmol) and DMAP (2.3 g, 18.78 mmol) in THF/DMF (2:1, 30 mL) for 48 h; compound **9** was obtained as a white solid (1.3 g, 73% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.14 (br s, 2H, 2x COOH), 9.49 (s, 1H, CHO), 6.47 (s, 1H, $H^{29\ pro\text{-}Z}$), 6.09 (s, 1H, $H^{29\ pro\text{-}E}$), 4.37 (dd, *J* = 11.3, 4.6 Hz, 1H, $H^{3\alpha}$), 3.25 (dd, *J* = 11.1, 4.8 Hz, 1H), 3.19 (d, *J* = 21.6 Hz, 2H), 2.52 – 2.46 (m, 4H), 2.20 – 2.12 (m, 2H), 1.97 – 1.89 (m, 1H), 1.81 – 1.77 (m, 2H), 1.58 – 1.11 (m, 17H), 0.91 (d, *J* = 7.2 Hz, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.78 (d, *J* = 3.6 Hz, 9H, 3x CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6): δ 195.6, 177.1, 175.0, 173.4, 171.6, 170.3, 156.3, 134.8, 79.9, 55.5, 54.6, 49.4, 41.9, 37.7, 37.4, 36.6, 36.1, 33.7, 31.6, 31.4, 29.2, 28.8, 27.6, 26.8, 23.3, 20.7, 20.5, 17.7, 16.4, 15.8, 15.6, 14.3, 14.1 ppm. HRMS (ESI): *m/z* calcd for $C_{34}H_{50}O_7$ $[M-H]^-$ = 569.3473, found $[M-H]^-$ = 569.3456.

Hemisuccinate of 21-oxoacid (11). Compound **11** was prepared by the General Procedure B using **3** (700 mg, 1.45 mmol), succinic anhydride (867 mg, 8.68 mmol) and DMAP (1.05 g, 8.68 mmol) in THF/DMF (2:1, 15 mL) for 40 h; compound **11** was obtained as a white solid (1.3 g, 73% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.51 (dd, *J* = 11.0, 5.5 Hz, 1H, $H^{3\alpha}$), 3.69 (s, 3H, COO**CH_3**), 3.22 – 3.16 (m, 1H), 2.70 – 2.60 (m, 4H), 2.49 – 2.43 (m, 2H), 2.13 (d, *J* = 18.6 Hz, 1H), 2.00 (dd, *J* = 12.5, 3.0 Hz, 1H), 1.92 – 1.23 (m, 9H, *overlap with solvent*), 1.21 (app s, 3H, CH_3), 1.20 (app s, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.83 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 207.5, 177.7, 175.1, 172.1, 172.0, 145.9, 81.5, 55.6, 53.3, 52.7, 51.2, 47.8, 45.4, 41.5, 38.7, 38.0, 37.3, 35.0, 33.9, 29.5, 29.3, 29.2, 28.1, 27.8, 25.3, 23.7, 21.4, 20.3, 20.2, 18.3, 17.0, 16.8, 16.7, 16.1 ppm. HRMS (ESI): *m/z* calcd $C_{35}H_{52}O_7$ for $[M+H]^+$ = 585.3786, found $[M+H]^+$ = 585.3796.

Hemisuccinate of 21,22-dioxoacid (12). Compound **12** was prepared by a previously published procedure.[7] ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.20 (br s, 1H, COOH), 4.40 (dd, *J* = 11.6, 4.7 Hz, 1H, $H^{3\alpha}$), 3.66 (s, 3H, COO**CH_3**), 3.37 – 3.30 (m, 1H, *overlap with solvent*), 2.72 – 2.69 (m, 1H), 2.52 – 2.44 (m, 4H, *overlap with solvent*), 2.25 – 2.22 (m, 1H), 1.97 – 1.87 (m, 3H), 1.74 – 1.31 (m, 14H), 1.19 (d, *J* = 3.5 Hz, 3H, CH_3), 1.18 (d, *J* = 3.5 Hz, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.79 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6): δ 194.4, 189.0, 173.4, 171.6, 170.5, 168.0, 149.2, 79.9, 58.1, 54.5, 53.5, 49.8, 45.6, 45.3, 41.0, 37.7, 37.4, 36.6, 33.9, 29.1, 28.7, 27.8, 27.5, 27.3, 26.4, 25.2, 23.3, 20.5, 19.9, 19.4, 17.7, 16.5, 16.4, 16.3, 15.6 ppm. HRMS (ESI): *m/z* calcd for $C_{35}H_{50}O_8$ $[M-H]^-$ = 597.3422, found $[M-H]^-$ = 597.3408.

General procedure C for preparation of glyoxalates. To a freshly degassed solution of starting material in CH_2Cl_2/CH_3OH (2:1) was added Pd/C (10%, 3.5 mol%) and H_2 was bubbled through the resulting reaction mixture for 20 min. The reaction was monitored with TLC (CH_2Cl_2/CH_3OH/AcOH = 10:1:0.1, v/v) which indicated its completion after 1h. The reaction mixture was diluted with CH_3OH (20 mL/ 0.83 mmol) and filtered through bed of Celite. The residual solvent was evaporated under reduced pressure and the crude product was purified by a flash chromatography (CH_2Cl_2/CH_3OH/AcOH 10:1:0.1, v/v).

Betulinic acid glyoxalate (8). Compound **8** was prepared by the General Procedure C using **7** (500 mg, 0.83 mmol), Pd/C (30 mg) in CH_2Cl_2/CH_3OH (2:1, 7.5 mL); compound **8** was obtained as a white solid (357 mg, 84% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.91 (br s, 1H, COOH), 4.65 (d, *J* = 2.0 Hz, 1H, $H^{29\ pro\text{-}Z}$), 4.53 (s, 1H, $H^{29\ pro\text{-}E}$), 4.50 (app s, 2H, O**CH_2**CO), 4.21 (br s, 1H, $H^{3\alpha}$), 2.94 (m, 1H), 2.90 – 2.84 (m, 1H), 2.18 – 2.13 (m, 2H), 1.92 – 1.86 (m, 1H), 1.83 – 1.75 (m, 1H), 1.62 (s, 3H, CH_3), 1.58 – 1.01 (m, 20H), 0.90 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.72 (s, 3H, CH_3), 0.62 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, DMSO-d_6): δ 174.6, 169.3, 150.1, 109.7, 76.7, 60.3, 55.7, 54.8, 49.9, 48.6, 46.5, 42.0, 38.5, 38.2, 37.4, 36.7, 36.2, 33.9, 31.3, 29.8, 28.9, 28.1, 27.1, 25.0, 20.4, 18.9, 17.9, 15.9, 15.8, 15.7, 14.3 ppm. HRMS (ESI): *m/z* calcd for $C_{32}H_{50}O_5$ $[M-H]^-$ = 513.3575, found $[M-H]^-$ = 513.3567.

Glyoxalate of pyrazine of betulinic acid (14). Compound **14** was prepared by the General Procedure C using **13** (500 mg, 0.78 mmol), Pd/C (27 mg) in CH_2Cl_2/CH_3OH (2:1, 7.5 mL); compound **14** was obtained as a white solid (210 mg, 49% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.45 (d, *J* = 2.3 Hz, 1H, pyrazine), 8.32 (d, *J* = 2.4 Hz, 1H, pyrazine), 4.71 (d, *J* = 1.9 Hz, 1H, $H^{29\ pro\text{-}Z}$), 4.59 (app s, 1H, $H^{29\ pro\text{-}E}$), 4.55 (app s, 2H, O**CH_2**CO), 2.92 (td, *J* = 11. 2, 10.9, 4.7 Hz, 1H, $H^{19\beta}$), 2.88 (d, *J* = 16.6 Hz, 1H, $H^{1\alpha}$), 2.46 (d, *J* = 16.6 Hz, 1H, $H^{1\beta}$), 2.28 – 2.20 (m, 2H), 1.93 (dd, *J* = 11.9, 8.1 Hz, 1H), 1.87 – 1.79 (m, 1H), 1.67 (s, 3H, CH_3), 1.67 – 1.28 (m, 15H), 1.24 (s, 3H, CH_3), 1.20 (s, 3H, CH_3), 1.17 – 1.05 (m, 2H), 1.00 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.72 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, DMSO-d_6): δ 174.7, 169.3, 158.5, 150.2, 150.1, 142.3, 141.7, 109.8, 60.2, 55.8, 52.0, 48.1, 47.8, 46.5, 42.1, 37.5, 36.2, 32.8, 31.3, 29.8, 29.0, 25.1, 24.0, 21.0,

19.5, 18.9, 15.8, 15.2, 14.4 ppm. HRMS (ESI): *m/z* calcd for $C_{34}H_{48}N_2O_4$ $[M-H]^-$ = 547.3530, found $[M-H]^-$ = 547.3527.

Methyl (*E*)-3-(1*H*-pyrrol-2-yl)acrylate (16). To a stirred solution of pyrrole-2-carbaldehyde **15** (1.5 g, 15.78 mmol) in CH_2Cl_2 (30 mL) was added methyl (triphenylphosphoranylidene)acetate (10.5 g, 31.57 mmol) in CH_2Cl_2 (50 mL). The resulting mixture was stirred at room temperature overnight. The residual solvent was evaporated and the crude oily product was purified by a flash chromatography (hexane/EtOAc = 2:1, v/v) to afford compound **16** as a pale pink solid (2 g, 84% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.65 (br s, 1H), 7.56 (d, *J* = 16.0 Hz, 1H), 6.94 – 6.93 (m, 1H), 6.58 – 6.57 (m, 1H), 6.31 – 6.28 (m, 1H), 6.00 (d, *J* = 16.0 Hz, 1H), 3.78 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.2, 134.5, 128.5, 122.6, 114.7, 111.2, 111.0, 51.8 ppm. HRMS (ESI): *m/z* calcd for $C_8H_9NO_2$ $[M+H]^+$ = 152.0706, found $[M+H]^+$ = 152.0705.

Methyl 3-(1*H*-pyrrol-2-yl)propanoate (17). To a freshly degassed solution of **16** (2 g, 13.25 mmol) in CH_3OH (20 mL) was added Pd/C (10% loading, 3.5 mol%, 500 mg) and H_2 was bubbled through the resulting reaction mixture for 20 min. The reaction was monitored with UHPLC-MS, which indicated its completion after 1h. The reaction mixture was diluted with CH_3OH (20 mL) and filtered through bed of Celite. The residual solvent was evaporated and the crude product was purified by a flash chromatography (hexane/EtOAc = 2:1, v/v) to afford compound **17** as a pale yellow oil (1.67 g, 90% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.51 (br s, 1H), 6.69 – 6.67 (m, 1H), 6.11 (dd, *J* = 5.7, 2.8 Hz, 1H), 5.92 – 5.91 (m, 1H), 3.71 (s, 3H), 2.92 (t, *J* = 6.8 Hz, 2H), 2.65 (t, *J* = 6.8 Hz, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.7, 131.1, 117.0, 108.2, 105.7, 52.0, 34.5, 22.7 ppm. HRMS (ESI): *m/z* calcd for $C_8H_{11}NO_2$ $[M+H]^+$ = 154.0863, found $[M+H]^+$ = 154.0863.

BODIPY-FL-methyl propanoate (18). To a stirred solution of **17** (1.67 g, 10.92 mmol) and 3,5-dimethylpyrrole-2-carboxaldehyde (1.54 g, 12.01 mmol) in CH_2Cl_2 (80 mL) was added dropwise $POCl_3$ (1.12 mL, 12.01 mmol) at 0°C and the reaction mixture was allowed to warm to ambient temperature. The reaction was monitored with UHPLC-MS, which indicated formation of dipyrromethane intermediate after 3 h. Then the reaction mixture was cooled to 0°C and DIPEA (8.1 mL, 49.14 mmol) was added dropwise, followed by stirring for 20 min at 0°C. $BF_3.OEt_2$ (5.4 mL, 43.68 mmol) was added subsequently and the reaction was stirred overnight at ambient temperature. The mixture was diluted with CH_2Cl_2 (50 mL) and brine (100 mL), filtered through bed of Celite, again diluted with brine (100 mL) and extracted with CH_2Cl_2 (5x 200 mL). Organic extracts were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by a flash chromatography (100% CH_2Cl_2) to obtain compound **18** as a dark green crystalline solid (2.13 g, 63% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.09 (s, 1H), 6.89 (d, *J* = 3.9 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 3.70 (s, 3H), 3.31 (t, *J* = 7.6 Hz, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 2.58 (s, 3H), 2.26 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.2, 160.8, 144.1, 135.5, 133.5, 128.3, 124.1, 120.7, 116.9, 52.0, 33.5, 30.0, 24.3, 15.2, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.23 (d, *J* = 31.4 Hz), -145.37 (d, *J* = 31.4 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{15}H_{17}BF_2N_2O_2$ $[M-H]^-$ = 305.1267, found $[M-H]^-$ = 305.1266. *Other spectral data were consistent with literature precedencies.*[3]

BODIPY-FL-propanoic acid (19). To a stirred solution of **18** (1.43 g, 4.67 mmol) in THF (30 mL) was added water (20 mL) and conc. HCl (10 mL) at 0°C. The reaction mixture was stirred at ambient temperature and monitored by UHPLC-MS, which indicated its completion after 52h. Then was the reaction mixture diluted with water (100 mL) and extracted with CH_2Cl_2 (3x 150 mL). Organic extracts were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. Following purification of the crude product by a flash chromatography (CH_2Cl_2/AcOH 100:1, v/v) to afford final compound **19** as a dark red crystalline solid (1.15 g, 85% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.30 (s, 1H), 7.70 (s, 1H), 7.09 (d, *J* = 4.0 Hz, 1H), 6.38 (d, *J* = 4.0 Hz, 1H), 6.31 (s, 1H), 3.10 – 3.05 (t, *J* = 7.1 Hz, 2H), 2.64 (t, *J* = 8.5 Hz, 2H), 2.47 (s, 3H), 2.26 (s, 3H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6): δ 173.4, 159.5, 156.9, 144.3, 134.5, 133.0, 128.8, 125.4, 120.4, 116.5, 32.3, 23.5, 14.5, 11.0 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.21 (d, *J* = 31.8 Hz), -145.36 (d, *J* = 31.8 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{14}H_{15}BF_2N_2O_2$ $[M-H]^-$ = 291.1111, found $[M-H]^-$ = 291.1105. *Other spectral data were consistent with published results.*[3]

Preparation of BAL resin. Aminomethyl polystyrene resin (1 g, loading 0.98 mmol/g) was swollen in CH_2Cl_2 (10 mL) for 30 min, washed with DMF (3x 10 mL), neutralized in DMF/piperidine (5:1, 10 mL) for additional 30 min and then again washed with DMF (5x 10 mL). Backbone amide linker (700 mg, 2.94 mmol) and HOBt (450 mg, 2.94 mmol) were dissolved in DMF/CH_2Cl_2 (1:1, 10 mL, v/v) and DIC (460 µL, 2.94 mmol) was added. The resulting solution was added to polypropylene fritted syringe with aminomethyl resin. The reaction slurry was shaken at ambient temperature overnight, followed by wash with DMF (3x 10 mL) and CH_2Cl_2 (3x 10 mL). Bromphenol blue test confirmed quantitative acylation of amino groups.

Procedure for reductive amination. BAL resin (1 g, loading 0.98 mmol/g) was swollen in CH_2Cl_2 (10 mL) for 30 min, then washed with dry THF (3x 10 mL) and dry DMF (3x 10 mL). The solution of 2-(2-aminoethoxy)ethanol (490 µL, 4.9 mmol) in DMF/AcOH (10:1, 10 mL, v/v) was added to polypropylene fritted syringe with BAL resin and it was shaken overnight at ambient temperature. Then, $NaBH(OAc)_3$ (210 mg, 2.94 mmol) in DMF/AcOH (20:1, 5 mL, v/v) was added portionwise to the reaction mixture during period of 4 h, followed by washing with DMF (5x 10 mL) and CH_2Cl_2 (3x 10 mL) and neutralization with DMF/TEA (10:1, 10 mL, v/v) for additional 30 min to obtain resin **20**. The loading was determined according to published procedure[8] (0.4 – 0.6 mmol/g).

Procedure for protection with Fmoc. Resin **20** (250 mg) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with CH_2Cl_2 (3x 5 mL). Fmoc-OSu (505 mg, 1.5 mmol) was dissolved in CH_2Cl_2 (3 mL) and added to polypropylene fritted syringe with the resin. The reaction slurry was shaken at ambient temperature overnight, followed by washing with CH_2Cl_2 (5x 5 mL). Analytical sample was cleaved from the resin and UHPLC-MS analysis confirmed the presence of desired product. MS (ESI): *m/z* $[M+H]^+$ = 328.

Procedure for acylation with BODIPY-FL propanoic acid. Resin **20** equipped with Fmoc (250 mg) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with DMF (3x 3 mL) and CH_2Cl_2 (3x 3 mL). BODIPY-FL propanoic acid **19** (220 mg, 0.75 mmol), HOBt (115 mg, 0.75 mmol) and DMAP (92 mg, 0.75 mmol) were dissolved in DMF/CH_2Cl_2 (1:1, 2.5 mL, v/v) and DIC (117 µL, 0.75 mmol) was added. The resulting solution was added to polypropylene fritted syringe the resin. The reaction slurry was shaken at ambient temperature overnight, followed by washing with DMF (10x 3 mL) and CH_2Cl_2 (10x 3 mL) to give resin **21**. Analytical sample was cleaved from the resin and UHPLC-MS analysis confirmed the presence of desired product. MS (ESI): *m/z* $[M-H]^-$ = 600.

General procedure D for deprotection of Fmoc. Resin **21** (250 mg) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with DMF (3x 3 mL). The freshly prepared solution of DMF/piperidine (20:1, 2.5 mL, v/v) was added to polypropylene fritted syringe with the resin. The reaction slurry was shaken at ambient temperature for 30 min, followed by wash with CH_2Cl_2 (3x 3 mL), THF (3x 3 mL), DMF (3x 3 mL), THF (3x 3mL) and CH_2Cl_2 (3x 3 mL).

Procedure for acylation with FAEEAA. Fmoc deprotected resin **21** (250 mg) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with DMF (3x 3 mL) and CH_2Cl_2 (3x 3 mL). [2-[2-(Fmoc-amino)ethoxy]ethoxy]acetic acid (334 mg, 0.9 mmol) and HOBt (137 mg, 0.9 mmol) were dissolved in DMF/CH_2Cl_2 (1:1, 3 mL, v/v) and DIC (140 µL, 0.9 mmol) was added. The resulting solution was added to polypropylene fritted syringe with the resin. The reaction slurry was shaken at ambient temperature overnight, followed by washing with DMF (10x 3 mL) and CH_2Cl_2 (10x 3 mL) which gave resin **22**. Analytical sample was cleaved from the resin and UHPLC-MS analysis confirmed the presence of desired product. MS (ESI): *m/z* $[M-H]^-$ = 745. The Fmoc was deprotected from the resin **22** by the general procedure to give resin **23**.

Procedure for acylation with acetic anhydride. Resin **23** (200 mg) was swollen in CH_2Cl_2 (2 mL) for 30 min and then washed with DMF (3x 2 mL) and CH_2Cl_2 (3x 2 mL). DMAP (92 mg, 0.75 mmol) was dissolved in CH_2Cl_2 (2.5 mL) and acetic anhydride (71 µL, 0.75 mmol) was added. The resulting solution was added to polypropylene fritted syringe with the resin. The reaction slurry was shaken at ambient temperature overnight, followed by wash with CH_2Cl_2 (5x 3 mL). Subsequent cleavage from the resin and UHPLC-MS analysis confirmed the presence of desired product **33**.

General procedure E for acylation with triterpene derivatives. Resin **23** (250 mg) was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with DMF (3x 3 mL) and CH_2Cl_2 (3x 3 mL). To each solution of premodified triterpenes **6, 8-12, 14** (0.9 mmol) in DMF/CH_2Cl_2 (1:1, 3 mL, v/v) was added DIC (70 µL, 0.45 mmol) and the resulting mixture was added to polypropylene fritted syringe with starting material. The reaction slurry was shaken at ambient temperature overnight, followed by wash with CH_2Cl_2 (10x 3 mL). The final compounds were cleaved according to General Procedure F.

General procedure F for cleavage from the resin. Cleavage of intermediates **24-31** and **33** in analytical scale (~5 mg) prior to analysis was carried out in CH_2Cl_2/TFA (10:1, 1 mL, v/v) for 30 min according to the General Information.

Cleavage of intermediates **24-31** and **33** in preparative scale (~250 mg): The corresponding resin was swollen in CH_2Cl_2 (3 mL) for 30 min and then washed with CH_2Cl_2 (5x 3 mL). Solution of CH_2Cl_2/TFA (10:1, 3 mL, v/v) was added to each polypropylene fritted syringe with resin. The reaction slurry was shaken at ambient temperature for 90 min (**28-30**) or 2 h (**24-27**, **31**, **33**) and then washed with CH_2Cl_2/TFA (10:1, 3x 3 mL, v/v) and CH_2Cl_2 (3x 3 mL). The cleavage cocktail with combined washes was evaporated under a stream of nitrogen, the crude products were dissolved in CH_3CN (3 mL) and purified by RP-HPLC to afford final compounds **24-33**.

BODIPY-FL-triterpene conjugate 24. Dark red crystalline solid (11.4 mg, 14% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19 (t, *J* = 5.6 Hz, 1H), 7.09 (s, 1H), 6.87 (d, *J* = 4.0 Hz, 1H), 6.37 (t, *J* = 5.5 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.11 (s, 1H), 4.73 (d, *J* = 1.5 Hz, 1H, $H^{29\ pro\text{-}Z}$), 4.60 (s, 1H, $H^{29\ pro\text{-}E}$), 4.48 – 4.44 (m, 1H), 4.26 – 4.24 (m, 2H), 4.01 (s, 2H), 3.69 – 3.65 (m, 5H), 3.60 – 3.56 (m, 5H), 3.52 – 3.47 (m, 5H), 3.43 (t, *J* = 5.6 Hz, 2H), 3.28 (t, *J* = 7.6 Hz, 2H), 3.02 – 2.97 (m, 1H, $H^{19\beta}$), 2.79 (t, *J* = 7.6 Hz, 2H), 2.55 (s, 3H), 2.48 (t, *J* = 6.9 Hz, 2H), 2.25 (s, 3H), 1.68 (s, 3H, CH_3), 1.64 – 1.15 (m, 23H, *overlap with solvent*), 0.96 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.81 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 181.0, 172.9, 172.7, 171.9, 170.5, 160.8, 156.9, 150.6, 144.2, 135.5, 133.4, 128.2, 124.1, 120.8, 116.8, 109.9, 81.4, 71.1, 70.8, 70.4, 70.2, 70.1, 69.1, 63.7, 56.5, 55.6, 50.6, 49.4, 47.1, 42.6, 41.0, 40.9, 39.5, 38.8, 38.5, 38.0, 37.3, 34.4, 33.4, 32.3, 31.1, 30.7, 30.0, 29.9, 28.2, 25.6, 24.1, 23.9, 21.1, 19.6, 18.4, 16.7, 16.4, 16.2, 15.2, 14.9, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.82 (d, *J* = 31.5 Hz), -145.96 (d, *J* = 31.5 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{58}H_{85}BF_2N_4O_{11}$ $[M-H]^-$ = 1061.6276, found $[M-H]^-$ = 1061.6275. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.20.

BODIPY-FL-triterpene conjugate 25. Dark red oil (10.0 mg, 10% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.52 (s, 1H, CHO), 7.17 (t, *J* = 5.2 Hz, 1H), 7.09 (s, 1H), 6.88 (d, *J* = 3.9 Hz, 1H), 6.36 (t, *J* = 5.4 Hz, 1H), 6.29 – 6.26 (m, 2H), 6.12 (s, 1H, $H^{29\ pro\text{-}Z}$), 5.91 (s, 1H, $H^{29\ pro\text{-}E}$), 4.47 – 4.43 (m, 1H), 4.27 – 4.24 (m, 2H), 4.01 (s, 2H), 3.69 – 3.64 (m, 5H), 3.61 – 3.55 (m, 5H), 3.52 – 3.47 (m, 5H), 3.45 – 3.43 (m, 2H), 3.29 (t, *J* = 7.6 Hz, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.64 (t, *J* = 6.9 Hz, 2H), 2.55 (s, 3H), 2.48 (t, *J* = 7.0 Hz, 2H), 2.25 (s, 3H), 2.21 – 2.09 (m, 2H), 2.00 – 1.95 (m, 2H), 1.73 – 1.16 (m, 19H, *overlap with solvent*), 0.93 (s, 3H, CH_3) 0.91 (s, 3H, CH_3), 0.82 (s, 6H, 2x CH_3), 0.81 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 195.2, 180.7, 172.9, 172.7, 171.9, 170.5, 160.8, 156.9, 144.3, 135.5, 133.4, 128.2, 124.1, 120.8, 116.8, 81.4, 71.1, 70.8, 70.4, 70.2, 70.1, 69.1, 63.7, 56.6, 55.6, 50.4, 42.5, 40.8, 39.5, 38.9, 38.4, 38.0, 37.3, 37.1, 34.4, 33.5, 32.1, 31.1, 30.1, 29.8, 28.2, 27.4, 24.1, 23.8, 21.0, 18.3, 16.7, 16.3, 16.2, 15.2, 14.8, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.85 (d, *J* = 31.8 Hz), -145.99 (d, *J* = 31.8 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{58}H_{83}BF_2N_4O_{12}$ $[M-H]^-$ = 1075.6069, found $[M-H]^-$ = 1075.5985. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.14.

BODIPY-FL-triterpene conjugate 26. Dark red solid (10.7 mg, 15% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.15 (t, *J* = 4.7 Hz, 1H), 7.09 (s, 1H), 6.88 (d, *J* = 3.9 Hz, 1H), 6.32 (t, *J* = 5.2 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 4.50 – 4.47 (m, 1H), 4.27 – 4.24 (m, 2H), 4.00 (s, 2H), 3.70 – 3.65 (m, 5H), 3.69 (s, 3H, COO**CH_3**) 3.61 – 3.56 (m, 5H), 3.52 – 3.48 (m, 5H), 3.45 – 3.42 (m, 2H), 3.29 (t, *J* = 7.6 Hz, 2H), 3.21 – 3.17 (m, 1H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.67 – 2.62 (m, 3H), 2.55 (s, 3H), 2.51 – 2.46 (m, 3H), 2.25 (s, 3H), 2.14 – 1.23 (m, 15H, *overlap with solvent*), 1.21 (d, *J* = 1.2 Hz, 3H, CH_3), 1.20 (d, *J* = 1.3 Hz, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.82 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 207.4, 175.1, 172.9, 172.7, 171.9, 171.8, 170.4, 160.8, 156.9, 145.9, 144.2, 135.5, 133.4, 128.2, 124.1, 120.8, 116.8, 81.2, 71.1, 70.8, 70.4, 70.2, 70.1, 69.1, 63.7, 55.6, 53.2, 52.7, 51.2, 47.8, 45.4, 41.5, 39.5, 38.8, 38.7, 38.0, 37.3, 35.0, 33.9, 33.5, 31.0, 29.9, 29.6, 28.1, 27.8, 25.3, 24.1, 23.8, 21.4, 20.3, 20.2, 18.3, 17.0, 16.8, 16.7, 16.1, 15.2, 14.3, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.71 (d, *J* = 34.5 Hz), -145.85 (d, *J* = 34.5 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{59}H_{85}BF_2N_4O_{12}$ $[M-H]^-$ = 1089.6225, found $[M-H]^-$ = 1089.6141. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.25.

BODIPY-FL-triterpene conjugate 27. Dark red solid (10.0 mg, 15% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.17 (t, *J* = 5.3 Hz, 1H), 7.09 (s, 1H), 6.88 (d, *J* = 3.9 Hz, 1H), 6.34 (t, *J* = 5.3 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 4.50 – 4.47 (m, 1H), 4.27 – 4.24 (m, 2H), 4.01 (s, 2H), 3.72 (s, 3H, COO**CH_3**), 3.69 – 3.65 (m, 4H), 3.61 – 3.56 (m, 4H), 3.53 – 3.48 (m, 4H), 3.46 – 3.42 (m, 2H), 3.39 – 3.33 (m, 1H), 3.31 – 3.28 (m, 2H), 2.81 – 2.76 (m, 2H), 2.67 – 2.64 (m, 2H), 2.56 (s, 3H), 2.51 – 2.48 (m, 2H), 2.25 (s, 3H), 2.11 – 1.91 (m, 6H), 1.76 – 1.32 (m, 14H, *overlap with solvent*), 1.28 (d, *J* = 6.9 Hz, 3H, CH_3), 1.25 (d, *J* = 6.9 Hz, 3H, CH_3), 1.06 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.83 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 194.5, 189.5, 176.3, 172.9, 172.1, 171.9, 171.2, 170.6, 168.3, 160.8, 156.8, 150.8, 144.3, 135.5, 133.4, 128.1, 124.1, 120.7, 116.7, 81.3, 81.1, 71.1, 70.8, 70.4, 70.2, 70.0, 69.1, 63.7, 58.6, 55.6, 53.6, 51.0, 46.3, 45.7, 41.7, 39.5, 38.7, 38.0, 37.3, 34.7, 33.4, 31.0, 29.9, 29.5, 29.0, 28.6, 28.1, 27.6, 26.1, 24.0, 23.7, 21.2, 20.0, 18.2, 17.0, 16.9, 16.7, 16.3, 15.1, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.04 (d, *J* = 34.4 Hz), -145.18 (d, *J* = 34.4 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{59}H_{83}BF_2N_4O_{13}$ $[M-H]^-$ = 1103.6018, found $[M-H]^-$ = 1103.5925. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.26.

BODIPY-FL-triterpene conjugate 28. Dark red solid (21.7 mg, 30% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, *J* = 2.3 Hz, 1H, pyrazine), 8.27 (d, *J* = 2.4 Hz, 1H, pyrazine), 7.08 (s, 1H), 7.07 – 7.04 (m, 1H), 6.87 (d, *J* = 4.0 Hz, 1H), 6.52 – 6.51 (m, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.11 (s, 1H), 4.75 (d, *J* = 1.6 Hz, 1H, $H^{29\ pro\text{-}Z}$), 4.64 (d, *J* = 1.3 Hz, 1H, $H^{29\ pro\text{-}E}$), 4.59 (d, *J* = 15.1 Hz, 1H, O**CH$_2$**CO), 4.53 (d, *J* = 15.1 Hz, 1H, O**CH$_2$**CO), 4.26 – 4.24 (m, 2H), 3.99 (s, 3H), 3.68 – 3.64 (m, 4H), 3.62 – 3.61 (m, 2H), 3.58 – 3.54 (m, 4H), 3.52 – 3.47 (m, 4H), 3.29 (t, *J* = 7.6 Hz, 2H), 3.09 (d, *J* = 16.6 Hz, 1H, $H^{1\alpha}$), 2.97 (dd, *J* = 11.0, 4.7 Hz, 1H), 2.79 (t, *J* = 7.4 Hz, 2H), 2.55 (s, 3H), 2.44 (d, *J* = 16.6 Hz, 1H, $H^{1\beta}$), 2.31 – 2.26 (m, 2H), 2.25 (s, 3H), 2.00 – 1.96 (m, 1H), 1.93 – 1.89 (m, 1H), 1.79 – 1.74 (m, 1H), 1.70 (s, 3H, CH_3), 1.68 – 1.32 (m, 13H, *overlap with solvent*), 1.29 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.79 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.8, 172.6, 170.1, 167.7, 160.8, 159.9, 156.9, 150.9, 150.7, 150.2, 144.2, 142.5, 141.5, 135.5, 133.4, 128.1, 124.0, 120.7, 116.7, 110.2, 71.1, 70.9, 70.4, 70.0, 69.9, 69.1, 63.7, 62.6, 56.8, 53.2, 49.5, 49.0, 48.8, 46.9, 42.7, 40.7, 39.7, 39.0, 38.8, 38.3, 37.1, 37.0, 33.5, 32.1, 31.7, 30.6, 29.9, 25.6, 24.2, 24.1, 21.6, 20.2, 19.6, 16.3, 15.8, 15.1, 14.8, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.04 (d, *J* = 31.8 Hz), -145.18 (d, *J* = 31.8 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{58}H_{81}BF_2N_6O_9$ $[M\text{-}H]^-$ = 1053.6126, found $[M\text{-}H]^-$ = 1053.6134. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.21.

BODIPY-FL-triterpene conjugate 29. Dark red solid (16.7 mg, 20% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.46 (app s, 1H, pyrazine), 8.28 (d, *J* = 2.4 Hz, 1H, pyrazine), 7.08 (s, 1H), 7.07 – 7.06 (m, 1H), 6.87 (d, *J* = 3.9 Hz, 1H), 6.57 (t, *J* = 5.1 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.11 (s, 1H), 4.75 (app s, 1H, $H^{29\ pro\text{-}Z}$), 4.64 (app s, 1H, $H^{29\ pro\text{-}E}$), 4.60 (d, *J* = 15.1 Hz, 1H, O**CH$_2$**CO), 4.53 (d, *J* = 15.1 Hz, 1H, O**CH$_2$**CO), 4.26 – 4.23 (m, 2H), 4.00 (s, 4H), 3.68 – 3.46 (m, 24H), 3.28 (t, *J* = 7.6 Hz, 2H), 3.09 (d, *J* = 16.7 Hz, 1H), 3.01 – 2.96 (m, 1H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.55 (s, 3H), 2.46 (d, *J* = 16.7 Hz, 1H), 2.30 – 2.27 (m, 2H), 2.25 (s, 3H), 2.00 – 1.93 (m, 2H), 1.77 (d, *J* = 11.5 Hz, 1H), 1.70 (s, 3H, CH_3), 1.68 – 1.34 (m, 14H, *overlap with solvent*), 1.30 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.80 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.8, 172.6, 170.1, 167.7, 160.8, 160.4, 157.1, 156.9, 151.0, 150.2, 144.2, 142.7, 135.5, 133.4, 128.2, 124.1, 120.7, 116.8, 110.2, 71.0, 70.9, 70.4, 70.1, 70.0, 69.0, 63.7, 62.6, 56.8, 53.1, 49.5, 48.9, 48.4, 46.9, 42.7, 40.7, 39.7, 39.0, 38.8, 38.3, 37.1, 37.0, 33.5, 32.2, 31.7, 30.6, 29.9, 25.6, 24.2, 24.1, 22.9, 21.6, 20.2, 19.6, 16.3, 15.8, 15.2, 14.8, 14.3, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.85 (d, *J* = 34.2 Hz), -145.99 (d, *J* = 34.2 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{64}H_{92}BF_2N_7O_{12}$ $[M+H]^+$ = 1200.6865, found $[M+H]^+$ = 1200.6848. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.30.

BODIPY-FL-triterpene conjugate 30. Dark red solid (8.5 mg, 12% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.09 (s, 1H), 7.07 – 7.06 (m, 1H), 6.88 (d, *J* = 3.9 Hz, 1H), 6.50 – 6.48 (m, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 4.73 (app s, 1H, $H^{29\ pro\text{-}Z}$), 4.60 (d, *J* = 2.0 Hz, 1H, $H^{29\ pro\text{-}E}$), 4.59 (d, *J* = 15.5 Hz, O**CH$_2$**CO), 4.52 (d, *J* = 15.1 Hz, 1H, O**CH$_2$**CO), 4.27 – 4.24 (m, 2H), 3.99 (s, 2H), 3.68 – 3.63 (m, 4H), 3.62 – 3.60 (m, 2H), 3.58 – 3.53 (m, 4H), 3.52 – 3.47 (m, 4H), 3.29 (t, *J* = 7.6 Hz, 2H), 3.18 (dd, *J* = 11.4, 4.8 Hz, 1H), 2.99 – 2.94 (m, 1H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.56 (s, 3H), 2.25 (s, 3H), 2.25 – 2.20 (m, 2H), 1.98 – 1.86 (m, 3H), 1.68 (s, 3H, CH_3), 1.65 – 1.15 (m, 19H, *overlap with solvent*), 0.96 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.75 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 174.8, 172.6, 170.1, 167.7, 156.9, 150.3, 144.3, 128.1, 124.1, 120.8, 116.8, 110.1, 79.2, 71.1, 70.9, 70.4, 69.9, 69.1, 63.7, 62.6, 56.8, 55.5, 50.7, 49.6, 47.0, 42.6, 40.9, 39.0, 38.9, 38.8, 38.3, 37.4, 37.1, 34.5, 33.5, 32.2, 30.6, 29.9, 28.2, 27.6, 25.7, 24.1, 21.0, 19.5, 18.5, 16.3, 16.2, 15.5, 15.2, 14.9, 11.5 ppm. ^{19}F {^{1}H} NMR δ -145.76 (d, *J* = 30.5 Hz), -145.90 (d, *J* = 30.5 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{56}H_{83}BF_2N_4O_{10}$ $[M\text{-}H]^-$ = 1019.6170, found $[M\text{-}H]^-$ = 1019.6171. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.17.

BODIPY-FL-triterpene conjugate 31. Dark red solid (15.2 mg, 21% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19 (t, *J* = 5.5 Hz, 1H), 7.09 (s, 1H), 6.88 (d, *J* = 4.0 Hz, 1H), 6.40 (t, *J* = 5.2 Hz, 1H), 6.27 (d, *J* = 4.0 Hz, 1H), 6.12 (s, 1H), 5.67 (app s, 1H, $H^{29\ pro\text{-}Z}$), 5.29 (app s, 1H, $H^{29\ pro\text{-}E}$), 4.27 – 4.24 (m, 2H), 4.03 (s, 2H), 3.71 – 3.66 (m, 4H), 3.64 – 3.50 (m, 10H), 3.29 (t, *J* = 7.6 Hz, 2H), 3.17 (dd, *J* = 11.0, 5.1 Hz, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.55 (s, 3H), 2.25 (s, 3H), 2.22 – 2.13 (m, 2H), 1.96 – 1.84 (m, 3H), 1.67 – 1.34 (m, 15H), 1.25 – 1.16 (m, 4H), 0.95 (s, 6H, 2x CH_3), 0.91 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.74 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 179.8, 172.6, 170.7, 169.1, 160.8, 156.9, 150.7, 144.3, 135.5, 133.4, 128.2, 127.9, 127.2, 125.1, 124.1, 120.8, 116.8, 79.1, 71.3, 70.8, 70.7, 70.3, 70.0, 69.1, 63.6, 56.5, 55.5, 50.9, 50.6, 42.6, 40.9, 39.4, 39.0, 38.9, 38.4, 37.4, 36.8, 34.5, 33.5, 32.9, 32.0, 29.8, 28.2, 27.5, 24.1, 21.1, 18.4, 16.3, 16.2, 15.6, 15.2, 14.9, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.75 (d, J = 35.6 Hz), -145.97 (d, J = 35.6 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{54}H_{79}BF_2N_4O_{10}$ $[M\text{-}H]^-$ = 991.5857, found $[M\text{-}H]^-$ = 991.5852. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.17.

BODIPY-FL-triterpene conjugate 32. Dark red oil (4.5 mg, 7% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.17 (t, *J* = 5.3 Hz, 1H), 7.09 (s, 1H), 6.88 (d, *J* = 3.8 Hz, 1H), 6.33 (t, *J* = 5.2 Hz, 1H), 6.27 (d, *J* = 3.8 Hz, 1H), 6.12 (s, 1H), 4.49 – 4.45 (m, 1H), 4.27 – 4.24 (m, 2H), 4.01 (s, 2H), 3.93 (s, 1H), 3.67 (dd, *J* = 9.2, 5.3 Hz, 4H), 3.61 – 3.56 (m, 4H), 3.53 – 3.48 (m, 4H), 3.46 – 3.42 (m, 2H), 3.29 (t, *J* = 7.5 Hz, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.66 – 2.62 (m, 2H), 2.55 (s, 3H), 2.50 – 2.47 (m, 2H), 2.25 (s, 3H), 1.85 (d, *J* = 13.4 Hz, 1H), 1.79 (d, *J* = 11.1 Hz, 1H), 1.72 – 1.18 (m, 24H, *overlap with solvent*), 1.02 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 0.82 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 180.1, 172.9, 172.6, 171.8, 170.4, 160.8, 156.9, 144.2, 135.5, 133.5, 128.2, 124.1, 120.8, 116.8, 109.9, 86.2, 81.3, 71.1, 70.8, 70.4, 70.2, 70.1, 69.1, 63.7, 55.8, 51.4, 46.9, 46.3, 40.8, 40.1, 39.5, 38.9, 38.8, 38.1, 37.4, 36.2, 33.9, 33.8, 33.5, 32.5, 32.1, 31.1, 30.1, 28.9, 28.1, 28.1, 26.7, 24.2, 24.1, 23.8, 21.1, 18.2, 16.8, 16.7, 15.7, 15.2, 13.8, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): δ -145.31 (d, *J* = 31.8 Hz), -145.45 (d, *J* = 31.8 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{58}H_{85}BF_2N_4O_{11}$ $[M\text{-}H]^-$ = 1061.6276, found $[M\text{-}H]^-$ = 1061.6278. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.20.

BODIPY-FL-2,11-dioxo-6,9,15-trioxa-3,12-diazaheptadecan-17-yl propionate 33. Dark red crystalline solid (2.49 mg, 11% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 (t, *J* = 5.5 Hz, 1H), 7.09 (s, 1H), 6.88 (d, *J* = 4.0 Hz, 1H), 6.29 – 6.27 (m, 2H), 6.12 (s, 1H), 4.27 – 4.24 (m, 2H), 4.01 (s, 2H), 3.69 – 3.66 (m, 4H), 3.61 – 3.57 (m, 4H), 3.53 – 3.47 (m, 4H), 3.45 – 3.41 (m, 2H), 3.29 (t, J = 7.6 Hz, 2H), 2.79 (t, *J* = 7.4 Hz, 2H), 2.56 (s, 3H), 2.25 (s, 3H), 1.98 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.7, 170.6, 170.5, 160.9, 156.9, 144.3, 135.5, 133.5, 128.2, 124.1, 120.1, 116.8, 71.2, 70.9, 70.5, 70.3, 70.1, 69.1, 63.7, 39.5, 38.8, 33.5, 24.1, 23.3, 15.2, 11.5 ppm. ^{19}F {^{1}H} NMR (471 MHz, $CDCl_3$): -145.64 (d, *J* = 35.6 Hz), -145.79 (d, *J* = 35.6 Hz) ppm. HRMS (ESI): *m/z* calcd for $C_{26}H_{37}BF_2N_4O_7$ $[M\text{-}H]^-$ = 565.2723, found $[M\text{-}H]^-$ = 565.2736. UV/Vis (CH_2Cl_2): λ_{max} = 507 nm; λ_{em} = 513 nm; Φ = 0.71.

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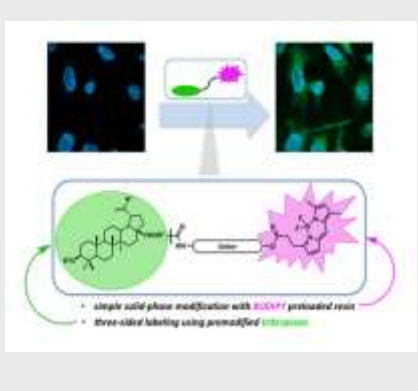

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Synthetic approach for rapid preparation of BODIPY conjugates and their use in imaging of cellular drug uptake and distribution