Steroids 102 (2015) 53–59

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Brassinosteroid-BODIPY conjugates: Design, synthesis, and properties

Magdalena Malachowska-Ugarte [a,b], Claudio Sperduto [a,b], Yuri V. Ermolovich [a], Alina L. Sauchuk [a], Michal Jurášek [b], Raisa P. Litvinovskaya [a], Darya Straltsova [c], Igor Smolich [c], Vladimir N. Zhabinskii [a,*], Pavel Drašar [b], Vadim Demidchik [c], Vladimir A. Khripach [a]

[a] Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Kuprevich Str., 5/2, 220141 Minsk, Belarus
[b] University of Chemistry and Technology, Technická 5, CZ-166 28 Praha 6, Czech Republic
[c] Department of Plant Cell Biology and Bioengineering, Biological Faculty, Belarusian State University, Minsk, Belarus

ARTICLE INFO

Article history:
Received 14 March 2015
Received in revised form 29 June 2015
Accepted 19 July 2015
Available online 22 July 2015

Keywords:
Brassinosteroids
BODIPY
Conjugates
Epibrassinolide
Epicastasterone
Castasterone

ABSTRACT

Three BS-BODIPY (brassinosteroids-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) conjugates were synthesized and their fluorescent and immunological properties were investigated. Two of the conjugates, having present all the functional groups characteristic of BS, were shown to be potentially useful as biological probes to study involvement of BS into physiological processes in living cells.

1. Introduction

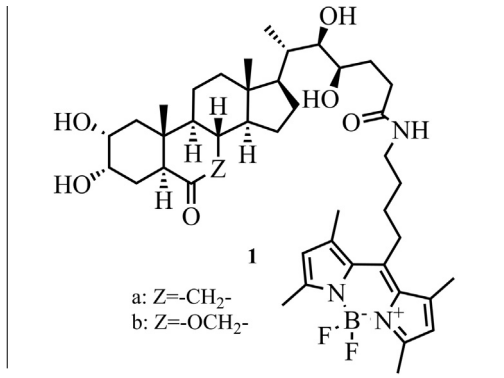
Tagging of biomolecules with a fluorescent label is one of the most common techniques employed for a wide range of multidisciplinary applications, such as analytical tools, pharmaceutical research, clinical diagnostics and visualizing normal physiological processes in living cells [1,2]. The corresponding derivatives of steroids have an increasingly important place among biological probes for such applications. A number of fluorescent conjugates of brassinosteroids (BS) were developed for studying these plant hormones [3–12]. However, all of the conjugates known to date have a common drawback in that they are derived by covalent binding of the fluorophore *via* one or more functional group(s) of the steroid molecule. Evidently, such a labeling would result in the formation of the fluorophore/biomolecule couple with properties differing from those of the parent BS. In this respect, the primary aim of this study was to design BS-fluorophore conjugates with a full set of functional groups of BS.

Another problem in the design of the required conjugates is the choice of the appropriate fluorophore. Apart from suitable fluorescence properties, the labeling fluorophore should not have considerable impact on the polarity, charge distribution, size and the stereochemistry of the conjugate. The literature analysis demonstrated that BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) based dyes that have emerged in the last two decades [13] have a number of advantages over other fluorophores, such as high extinction coefficient and fluorescence quantum yield, narrow emission bandwidth, relatively long excited-state lifetimes, and small dependence of fluorescence properties on pH and polarity [14,15]. In studies of steroids, just to mention a few examples, the corresponding conjugates were employed as suitable tools for visualizing intracellular localization and tracking of cholesterol [16,17], dehydroepiandrosterone [18], nandrolone and related compounds [19], for revealing subcellular localization of the vitamin D receptor and measuring its content in single cells [20], determining the distribution of estradiol receptors [21], and the human progesterone receptor imaging in live cells [22]. Therefore, the combination of above objectives and constraints led us to consider structures **1a**, **1b**, and **2** (Fig. 1) as a platform for the development of biological probes to study involvement of BS into physiological processes in living cells.

Abbreviations: BS, brassinosteroids; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; $(DHQD)_2AQN$, hydroquinidine anthraquinone-1,4-diyl diether; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; DIPEA, *N,N*-diisopropylethylamine; HOBt, 1-hydroxybenzotriazole hydrate.

* Corresponding author.
E-mail address: vz@iboch.bas-net.by (V.N. Zhabinskii).

http://dx.doi.org/10.1016/j.steroids.2015.07.002
0039-128X/



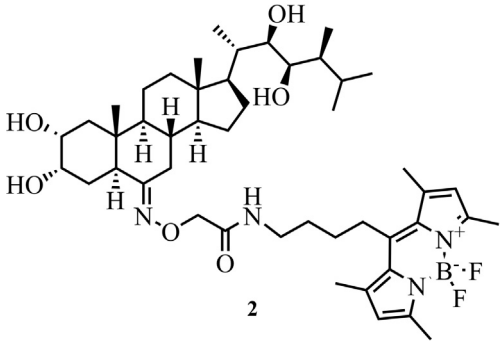


Fig. 1. Chemical structure of brassinosteroid-BODIPY conjugates **1a**, **1b**, and **2**.

2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were obtained using a Bruker AVANCE 500 (Bruker Biospin, Rheinstetten, Germany) spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shift values are given in δ (ppm) relative to the residual solvent peaks: δ_H 7.58 and δ_C 135.91 for C_5D_5N; δ_H 7.26 and δ_C 77.00 for $CDCl_3$, and coupling constants are reported in Hz. High resolution ESIMS were obtained on Thermo Fischer Scientific LTQ Orbitrap Velos. Light absorption and fluorescence emission spectra were measured on a Cary Eclipse spectrofluorimeter and analysed using standard Varian software (Varian, Australia). Chemicals were purchased from Aldrich and Fluka and used as received. Epicastasterone **3a** and epibrassinolide **3b** were obtained from Mikonik Technologies (Belarus). All solvents were purified according to standard methods [23]. Reactions were monitored by TLC using aluminum sheets, silica gel 60 F_{254} precoated (Merck Art. 5715). Column chromatography was carried out on Kieselgel 60 (Merck Art. 7734).

2.1.1. (E)-2α,3α-Isopropylidenedioxy-27-nor-5α-cholest-22-en-6-on-26-oic acid ethyl ester (**6a**)

To a cooled to −78 °C solution of (20*S*)-2α,3α-isopropylidenedioxy-20-formyl-5α-pregnan-6-one (**4a**) (525 mg, 1.3 mmol, prepared from epicastasterone (**3a**) according to the procedure described in [24]), 1 M vinylmagnesium bromide solution in THF (1.69 mL, 1.69 mmol) was added dropwise keeping the temperature below −60 °C. The mixture was stirred at −78 °C for 1.5 h, then NH_4Cl (193 mg, 3.61 mmol) was added and the temperature was raised to ambient. The mixture was diluted with water (7 mL) and EtOAc (4.2 mL). The water phase was separated and extracted with EtOAc (2 × 1 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to give 570 mg of crude **5a**. It was dissolved in toluene (27 mL), then triethyl orthoacetate (1.33 mL, 7.28 mmol) and acetic acid (0.1 mL, 1.6 mmol) were added. The mixture was refluxed under argon for 4 h. After cooling to room temperature, pyridine (0.41 mL) was added and solvents were evaporated *in vacuo*. The residue was chromatographed on silica gel (petroleum ether–EtOAc = 9:1) to give ester **6a** (372 mg, 57%) as an oil. ^{1}H NMR ($CDCl_3$) δ: 0.65 (s, 3H, C18-H), 0.66 (s, 3H, C19-H), 0.99 (d, J = 6.6 Hz, 3H, C21-H), 1.25 (t, J = 7.2 Hz, 3H, $C\underline{H}_3CH_2O$–), 1.33 (s, 3H, Me_2C<), 1.50 (s, 3H, Me_2C<), 4.05–4.15 (m, 3H, $CH_3C\underline{H}_2O$– and C2-H), 4.24–4.30 (m, 1H, C3-H), 5.29 (m, 2H, C22- and C23-H). ^{13}C NMR ($CDCl_3$) δ: 12.1, 12.7, 14.2, 20.5, 21.1, 22.5, 23.9, 26.5, 27.8, 28.2, 28.6, 34.5, 37.5, 39.2, 39.8, 41.1, 42.5, 42.7, 46.9, 51.5, 53.4, 55.6, 56.7, 60.2, 72.1, 72.3, 107.9, 125.6, 137.8, 173.2, 211.5.

2.1.2. (E)-2α,3α-Isopropylidenedioxy-27-nor-B-homo-7-oxa-5α-cholest-22-en-6-on-26-oic acid ethyl ester (**6b**)

The title compound (270 mg) was prepared as a colorless oil in 53% yield starting from (20*S*)-2α,3α-isopropylidenedioxy-20-formyl-B-homo-7-oxa-5α-pregnan-6-one (**4b**) (available from epibrassinolide (**3b**) according to [25]) as described above for the preparation of **6a**. ^{1}H NMR ($CDCl_3$) δ: 0.69 (s, 3H, C18-H), 0.87 (s, 3H, C19-H), 0.97 (d, J = 6.6 Hz, 3H, C21-H), 1.24 (t, J = 7.2 Hz, 3H, $C\underline{H}_3CH_2O$–), 1.30 (s, 3H, Me_2C<), 1.51 (s, 3H, Me_2C<), 3.27 (dd, J = 10.1, 4.4 Hz, 1H, C5-H), 4.00–4.16 (m, 5H, $CH_3C\underline{H}_2O$–, C2- and C7-H), 4.30–4.41 (m, 2H, C2- and C3-H), 5.19–5.38 (m, 2H, C22- and C23-H). ^{13}C NMR ($CDCl_3$) δ: 12.3, 14.2, 19.6, 20.5, 22.9, 23.6, 24.5, 26.5, 27.6, 27.8, 28.2, 33.5, 34.4, 35.9, 39.3, 39.6, 39.8, 40.2, 43.0, 52.0, 54.7, 55.5, 60.2, 71.2, 72.4, 73.1, 107.5, 125.7, 137.7, 173.2, 176.6.

2.1.3. (E)-2α,3α-Dihydroxy-27-nor-5α-cholest-22-en-6-on-26-oic acid (**8a**)

The ester **6a** (190 mg, 3.79 mmol) was dissolved in THF (2.23 mL), and then water (0.63 mL) and $LiOH{\cdot}H_2O$ (48 mg, 1.13 mmol) were added to the resulting solution. The mixture was stirred at 65 °C for 7 h under argon, then acetic acid (1.6 mL) and water (0.4 mL) were added and the resulting solution was left stirring at 70 °C for 4 h. After evaporation of solvent, the crude product was chromatographed on silica gel ($CHCl_3$–MeOH = 10:0.5) to afford the free acid **8a** (100 mg, 61%) as crystalline white solid. Mp 188.5–191.5 °C (acetone). ^{1}H NMR (C_5D_5N) δ: 0.58 (s, 3H, C18-H), 0.84 (s, 3H, C19-H), 1.02 (d, J = 6.6 Hz, 3H, C21-H), 3.14 (dd, J = 12.4, 2.6 Hz, 1H, C5-H), 4.07 (ddd, J = 11.0, 5.1, 2.7 Hz, 1H, C2-H), 4.44 (d, J = 2.7 Hz, 1H, C3-H), 5.43 (dd, J = 15.1, 8.6 Hz, 1H, C22- or C23-H), 5.55 (dt, J = 15.1, 6.4 Hz, 1H, C23- or C22-H). ^{13}C NMR (C_5D_5N) δ: 12.7, 14.2, 21.1, 21.8, 24.4, 28.3, 29.0, 29.0, 35.5, 38.1, 39.9, 40.6, 41.7, 43.0, 43.3, 47.3, 51.9, 54.3, 56.3, 57.1, 68.9, 69.4, 127.0, 175.9, 212.1. HRMS (ESI^-): calcd. for $C_{26}H_{39}O_5$ $[M-H]^-$ 431.2798, found 431.2780.

2.1.4. (22E)-2α,3α-Dihydroxy-27-nor-B-homo-7-oxa-5α-cholest-22-en-6-on-26-oic acid (**8b**)

The title compound (73 mg) was prepared as a white solid in 86% yield starting from ester **6b** as described above for the preparation of **8a**. ^{1}H NMR (C_5D_5N) δ: 0.56 (s, 3H, C18-H), 0.99 (d, J = 6.6 Hz, 3H, C21-H), 1.05 (s, 3H, C19-H), 3.61 (dd, J = 12.0, 4.4 Hz, 1H, C5-H), 4.00–4.13 (m, 3H, C2- and C7-H), 4.44 (br.s, 1H, C3-H), 5.40 (dd, J = 15.2, 8.6 Hz, 1H, C22- or C23-H), 5.54 (dt, J = 14.8, 6.4 Hz, 1H, C23- or C22-H). ^{13}C NMR (C_5D_5N) δ: 12.4, 16.3, 21.1, 22.8, 25.2, 28.9, 29.0, 33.5, 35.5, 38.9, 39.9, 40.0, 40.6, 42.1, 43.0, 43.2, 51.8, 56.2, 58.7, 68.8, 69.1, 70.6, 138.1, 175.9, 177.0. HRMS (ESI^-): calcd. for $C_{26}H_{39}O_6$ $[M-H]^-$ 447.2747, found 447.2757.