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Short Note

28-[1-(3-(Propionyloxy)propyl)-1*H*-1,2,3-triazol-4-yl]carbonylbetulin

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Abstract: Betulin has a broad spectrum of biological and pharmacological properties, such as anticancer, antibacterial, antifungal, and antiviral. Unfortunately, the low bioavailability makes it difficult to use in medicine. The introduction of a triazole ring to the betulin structure leads to the obtainment of new compounds with higher activity and better bioavailability. The title compound was obtained from the triazole derivative of betulin by conversion of the hydroxyl group to an ester moiety in the Steglich reaction. The chemical structure of the hybrid was characterized by nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, HSQC, HMBC) and HRMS spectroscopy.

Keywords: betulin; triazole; NMR; spectroscopic methods

Citation: Kadela-Tomanek, M.; Chrobak, E.; Bębenek, E. 28-[1-(3-(Propionyloxy)propyl)-1*H*-1,2,3-triazol-4-yl]carbonylbetulin. *Molbank* **2022**, *2022*, M1487. https://doi.org/10.3390/M1487

Academic Editor: R. Alan Aitken

Received: 26 September 2022
Accepted: 7 November 2022
Published: 9 November 2022

1. Introduction

Betulin, a pentacyclic triterpene of the lupane-type, has a wide range of biological and pharmacological properties. This compound is characterized by poor water solubility, which reduces its bioavailability. Betulin can be used as a building block for the synthesis of new derivatives by converting the hydroxyl groups at the C3 and C28 positions or by introducing modifications to the isopropenyl group attached to a five-membered ring (Figure 1) [1–6].

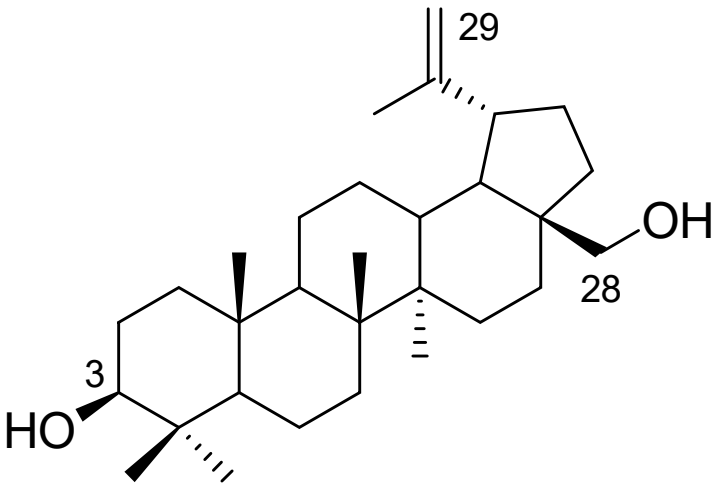


Figure 1. Chemical structure of betulin.

Betulin undergoes reactions characteristic of alcohols, such as esterification and oxidation [7]. In the synthesis of betulin esters, Steglich esterification is usually used. This reaction takes place under mild conditions and does not require high temperatures. The synthesis of esters by this method is performed with *N*,*N*'-dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)pyridine (DMAP). The addition of DMAP accelerates the reaction and reduces the formation of by-products associated with the migration of the acyl group [8,9].

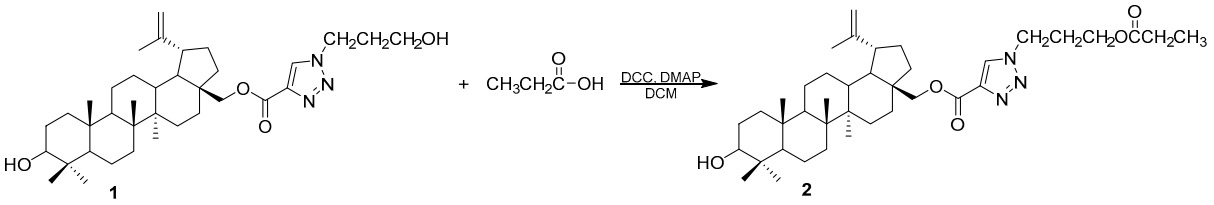
The modification of betulin is also associated with the introduction of the triazole ring. The triazole ring occurs in the chemical structure of compounds that exhibit anticancer, anti-inflammatory, anti-tuberculosis, antibacterial, antifungal, antioxidant, and analgesic effects. The possibility of introducing various substituents into the triazole ring is important for modulating the biological properties of the triazoles and allows their wide application [10].

It is supposed that hybrid compounds can reduce side effects and overcome drug resistance. Hybrids with several pharmacophores may also exhibit different mechanisms of biological action. The combination of the triazole system with other anticancer pharmacophores may lead to the formation of new derivatives of low toxicity and greater effectiveness in the treatment of drug-resistant neoplasms [11]. For example, betulin-triazole hybrids often possess higher biological activity than betulin. It has been shown that triazole derivatives of betulin are cytotoxic to cells of lymphoblastic leukemia, cervical cancer, ovarian cancer, breast cancer, colon cancer, prostate cancer, lung cancer, and melanoma [12–16]. Moreover, in the group of triazole derivatives of pentacyclic triterpenes, there are compounds with antimicrobial, antiviral, and neuroprotective effects [17–19].

The study describes the synthesis of new triazole derivatives of betulin. The structure of the title compound was characterized by homo- (^{1}H and ^{13}C NMR) and heteronuclear (HSQC and HMBC) magnetic resonance spectroscopy and HRMS spectrometry.

2. Results and Discussion

The triazole derivative **1** was synthesized from betulin in a multi-step reaction which is described in our earlier papers [13,16]. The treatment of compound **1** with propanoic acid in the presence of N,N′-dicyclohexylcarbodiimide (DCC), and 4-dimethylaminopyridine (DMAP) in dichloromethane (DCM) leads to ester **2** (Scheme 1).



Scheme 1. Synthesis of 28-[1-(3-(propionyloxy)propyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin **2**.

The crude product was purified by column chromatography. Compound **2** was obtained with a high yield (79%). The structure of the title compound **2**, which consists of three moieties, betulin, triazole linker, and the ester group (Figure 2), was characterized by 1D (^{1}H and ^{13}C) and 2D (HMBC and HSQC) NMR and the HR-MS spectra.

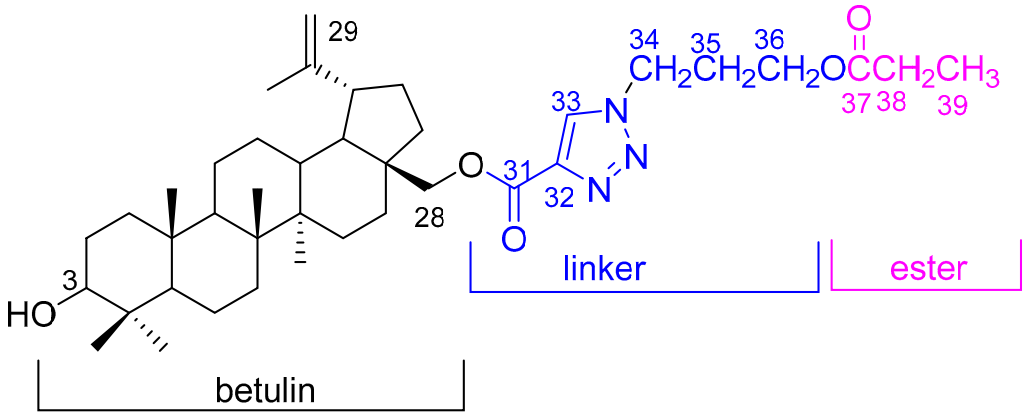


Figure 2. Chemical structure of compound **2**.

The chemical shift of the main hydrogen and carbon atoms in the betulin moiety was assigned based on the literature data [20,21]. The ^{1}H NMR spectra of the betulin scaffold

indicated the presence of six methyl groups at δ_H 0.78, 0.85, 0.99, 1.01, 1.08, and 1.72 ppm. The chemical shift of the proton at the C3 position was observed at δ_H 3.21 ppm. Signals located at δ_H 4.15 ppm and 4.59 ppm were assigned to the protons of the methylene group in position C28. The singlets at δ_H 4.62 ppm and 4.73 ppm indicated the presence of protons at a C29 position (Table 1, Figure 3). In the ^{13}C NMR spectrum of the title compound, the signal at δ_C 79.0 ppm was assigned to the C3 carbon atom, which is characteristic of betulin derivatives containing a hydroxyl group in this position. The signals at δ_C 63.6 ppm and 110.0 ppm were assigned to carbon atoms at C28 and C29, respectively (Table 1, Figure 3).

Table 1. The selected chemical shifts (^{1}H NMR and ^{13}C NMR spectra) and correlations of proton–carbon (HSQC and HMBC experiments) for derivative **2**.

Proton	^{1}H NMR δ [ppm]	HSQC	Carbon	^{13}C NMR δ [ppm]	HMBC
H39	1.16	H39(1.16)-C39(9.1)	C39	9.1	H39(1.16)-C38(27.4) H39(1.16)-C37(174.2)
H38	2.36	H38(2.36)-C38(27.4)	C38	27.4	H38(2.36)-C39(9.1) H38(2.36)-C37(174.2)
-	-	-	C37	174.2	C37(174.2)-H39(1.16) C37(174.2)-H38(2.36) C37(174.2)-H36(4.15)
H36	4.15	H36(4.15)-C36(60.6)	C36	60.6	H36(4.15)-C37(174.2) H36(4.15)-C35(29.4)
H35	2.32	H35(2.32)-C35(29.4)	C35	29.4	H35(2.32)-C34(47.7) H35(2.32)-C36(60.6)
H34	4.54	H34(4.54)-C34(47.7)	C34	47.7	H34(4.54)-C33(127.5) H34(4.54)-C36(60.6) H34(4.54)-C35(29.4)
H33	8.10	H33(8.10)-C33(127.5)	C33	127.5	H33(8.10)-C32(140.3)
-	-	-	C32	140.3	C32(140.3)-H33(8.10)
-	-	-	C31	161.1	C31(161.1)-H28b(4.59) C31(161.1)-H28a(4.15)
H29a	4.73	H29a(4.73)-C29(110.0)	C29	110.0	H29a(4.73)-C_{bet}(47.7) H29a(4.73)-C_{bet}(19.1)
H29b	4.62	H29a(4.62)-C29(110.0)	C29	110.0	H29b(4.62)-C_{bet}(47.7) H29b(4.62)-C_{bet}(19.1)
H28a	4.59	H28a(4.59)-C28(63.6)	C28	63.6	H28a(4.59)-C31(161.1)
H28b	4.15	H28a(4.16)-C28(63.6)	C28	63.6	H28b(4.15)-C31(161.1)
H3	3.21	H3(3.21)-C3(79.0)	C3	79.0	H3(3.21)-C_{bet}(28.0) H3(3.21)-C_{bet}(15.4)

The signals of the triazole linker and the ester moiety were assigned on the basis of the HMBC correlation spectra (Table 1, Figure 4). The HMBC shows that the methyl group at the C39 position correlated with C38 and C37. The carbon atoms at the C37 and C39 positions were identified based on their correlation with the proton at the C38 position. Furthermore, the C37 carbon (δ_C 174.2 ppm) was correlated with the H39, H38, and H36 protons, respectively. The spectrum also showed the correlation of CH_2 in position C34 with the carbon atom in position C35 (δ_C 29.4 ppm) and C33 (δ_C 127.5 ppm), respectively. The correlation between the proton signal at C33 (δ_C 8.10 ppm) and the carbon signal at C32 (δ_C 140.3 ppm) was also observed. The carbonyl group at the C31 position had a correlation with the carbon atom at the C28 (δ_C 63.6 ppm) position (Table 1, Figure 4).

The exact mass of the $[M + Na]^+$ ion, determined by ESI-HRMS, was found to be 674.4505 (674.4509 as calculated for $C_{39}H_{61}N_3O_5Na^+$).

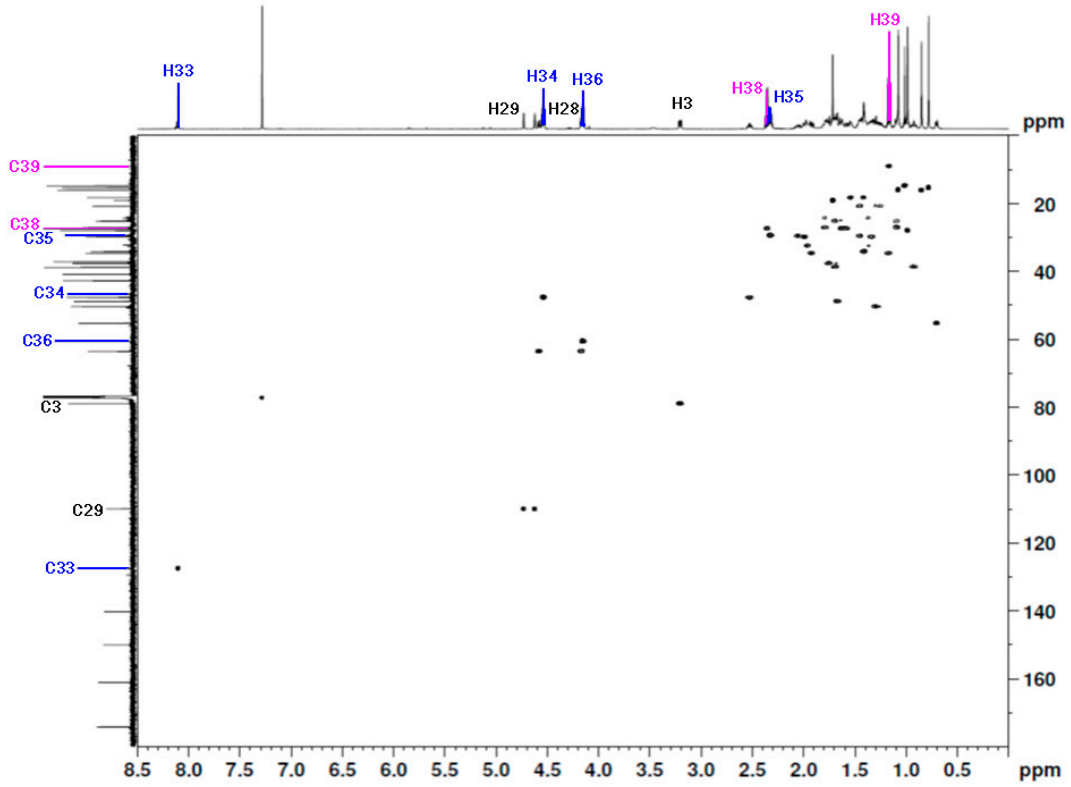


Figure 3. The ^{1}H-^{13}C HSQC spectrum (600 MHz, $CDCl_3$) of 28-[1-(3-(propionyloxy)propyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin **2**.

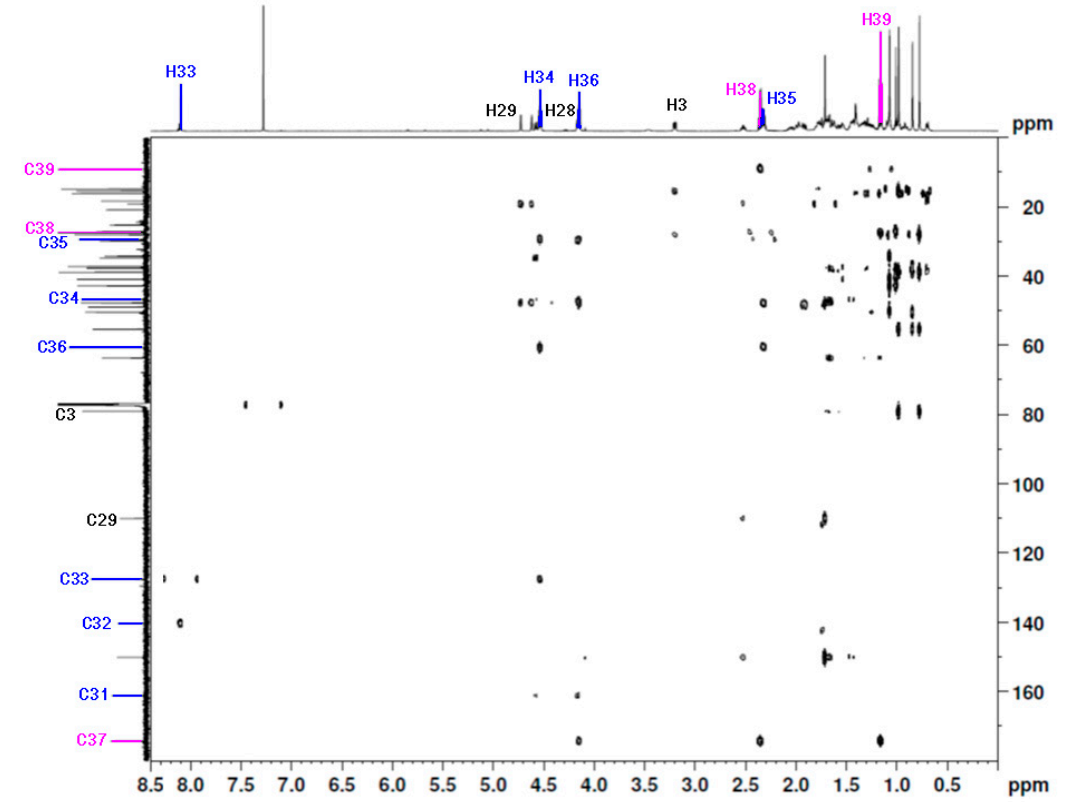


Figure 4. The ^{1}H-^{13}C HMBC spectrum (600 MHz, $CDCl_3$) of 28-[1-(3-(propionyloxy)propyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin **2**.

3. Materials and Methods

3.1. General Method

All reagents were purchased from Sigma-Aldrich (Darmstadt, Germany). The 28-[1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin **1** was obtained using the literature method [16]. The ^{1}H and ^{13}C NMR spectra were acquired on the Bruker Avance 600 spectrometer (Brucker Analytische Messtechnik GmbH, Rheinstetten, Germany) at 600 MHz and 150 MHz, respectively, as well as the HMBC and HSQC NMR spectra. The compound was dissolved in a deuterated chloroform ($CDCl_3$) solvent. Chemical shifts (δ) were reported in ppm and J values in Hz. Multiplicity was designated as singlet (s), doublet (d), triplet (t), and multiplet (m). The protons of betulin, the triazole linker, and the ester moiety were denoted by the appropriate indices as beta, linker, and ester. High-resolution mass spectra were measured on the Bruker Impact II instrument (Brucker Analytische Messtechnik GmbH, Rheinstetten, Germany). Melting points were measured by the Electrothermal IA 9300 melting point apparatus.

3.2. Synthesis of 28-[1-(3-(Propionyloxy)propyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin **2**

The 28-[1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin **1** 0.60 g (1 mmol) and propanoic acid 84 µL (0.310 mmol, 0.08 g) were dissolved in 4 mL of dichloromethane (DCM) and cooled to −10 °C. At this temperature, the mixture of 0.116 g DCC (0.605 mmol) and DMAP 0.005 g (0.080 mmol) in the dichloromethane (1 mL) was dropped. The reaction mixture was stirred overnight at room temperature. Then, the precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$:EtOH, 40:1, *v*/*v*). The desired product was a white crystalline solid (mp. 230–232 °C, yield 79%, 0.516 g, 0.79 mmol).

^{1}H NMR (600 MHz, $CDCl_3$) δ, ppm: 0.65 (d, J = 9.36 Hz, 1H, H_{5bet}), 0.78 (s, 3H, CH_{3bet}), 0.85 (s, 3H, CH_{3bet}), 0.99 (s, 3H, CH_{3bet}), 1.01 (s, 3H, CH_{3bet}), 1.08 (s, 3H, CH_{3bet}), 1.16 (t, J = 7.56 Hz, 3H, CH_{3ester}), 1.72 (s, 3H, CH_{3bet}), 0.90–2.09 (m, 25H, CH_{bet}, CH_{2bet}), 2.32 (m, 2H, $CH_{2linker}$); 2.36 (m, CH_{2ester}), 2.53 (m, 1H, $H19_{bet}$), 3.21 (m, 1H, $H3_{bet}$), 4.15 (m, 3H, $CH_{2linker}$, $H28_{bet}$), 4.54 (t, J = 6.96 Hz, 2H, $CH_{2linker}$); 4.59 (d, J = 10.8 Hz, 1H, $H28_{bet}$), 4.62 (s, 1H, $H29_{bet}$), 4.73 (s, 1H, $H29_{bet}$), 8.10 (s, 1H, CH_{linker}) (Figure S1), ^{13}C NMR (150 MHz, $CDCl_3$) δ, ppm: 9.1, 14.8, 15.4, 16.1, 18.3, 20.8, 24.3, 27.1, 27.4, 28.0, 29.4, 29.6, 29.8, 34.2, 34.7, 37.2, 37.7, 38.7, 38.9, 40.9, 42.8, 46.7, 47.7, 48.9, 50.4, 55.3, 60.6, 63.6, 79.0, 110.0, 127.5, 129.5, 140.3, 150.1, 161.1, 174.2 (Figure S2). ESI-HRMS *m*/*z* $[M + Na]^+$ calcd for $C_{39}H_{61}N_3O_5Na^+$ 674.4509, found 674.4505 (Figure S3).

Supplementary Materials: The following supporting information are available online, Figure S1: ^{1}H NMR spectrum (600 MHz, CDCl3) of 28-[1-(3-(propionyloxy)propyl)-1H-1,2,3-triazol-4-yl] carbonylbetulin; Figure S2: ^{13}C NMR spectrum (150 MHz, CDCl3) of 28-[1-(3-(propionyloxy)propyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin; Figure S3: ESI-HRMS spectrum of 28-[1-(3-(Propionyloxy)propyl)-1H-1,2,3-triazol-4-yl]carbonylbetulin.

Author Contributions: Conceptualization, methodology, writing—original draft preparation, M.K.-T.; formal analysis, writing—original draft preparation, E.C. and E.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Medical University of Silesia in Katowice, Poland. Grants No PCN-1-042/K/2/F and PCN-1-044/K/2/F.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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