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N-Propargylation of Indolo-Triterpenoids and Their Application in Mannich Reaction

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Abstract: The introduction of the alkynyl moiety to the triterpenic core through a linkage to the indole nitrogen is described. The reaction of *N*-propargylindoles with *N*-methylpiperazine using Mannich reaction led to propargylaminoalkynyl-triterpenoids, whose structures were established by NMR spectroscopy.

Keywords: triterpenoids; indoles; *N*-propargylation; alkyne; Mannich reaction

1. Introduction

During the last few years, the synthetic transformation of natural compounds to prepare biologically active analogues has become a topical course of bioorganic chemistry. Triterpenoids are a large group of natural compounds that possess a broad-spectrum pharmacological activity and represent a biologically active scaffold for chemical transformations because of several key positions available on the molecule [1,2]. One of the trending topics in the chemistry of triterpenoids is the synthesis of various heterocyclic derivatives by the condensation with A-ring, modification of carboxylic group and C3 position [3].

Among the *N*-heterocycles, the indole ring system is an important structural component in many pharmaceutical agents [4]. 2,3-Indolo-12-oxo-oleanolic acid showed significant inhibition activity towards protein tyrosine phosphatase 1B [5]. The IC_{50} value of ursolic acid modified with 5-methylindole moiety was comparable to doxorubicin [6], and the chloro-derivative of indolo-betulinic acid was found to be very active towards several cancer cell lines [7]. According to previous reports, some indolo-fused lupanes showed α-glucosidase inhibitory activity [8–10]. 2,3-Indolouvaol and 2,3-indolo-28-cyanoethoxybetulin showed antiproliferative activity in vitro towards leukemia, lung, and colon cancer cells [11].

The indole cycle can be converted into other ring systems leading to further privileged structures. According to our recent studies, the oxidized 28-oxo-indolo-allobetulone derivatives possess antiviral activity [12]. Oxidation of an aromatic moiety by H_2O_2 provides the ursane indoloquinone formation [13].

At the same time, there are no reports about the synthesis of *N*-substituted indole-fused triterpenoids, which in turn opens up the possibility for obtained of new conjugates. For example, in the last decade one of the priority topics in the chemistry of triterpenoids is the synthesis of various alkynyl derivatives with subsequent modification by click-chemistry to produce biologically active compounds bearing a 1,2,3-triazolyl fragment [14,15]. The conjugates obtained by click or Mannich reaction of C-28-propargyl amide or ester derivatives of 2,3-indolo-triterpenic acid possess the anticancer activity [16,17].

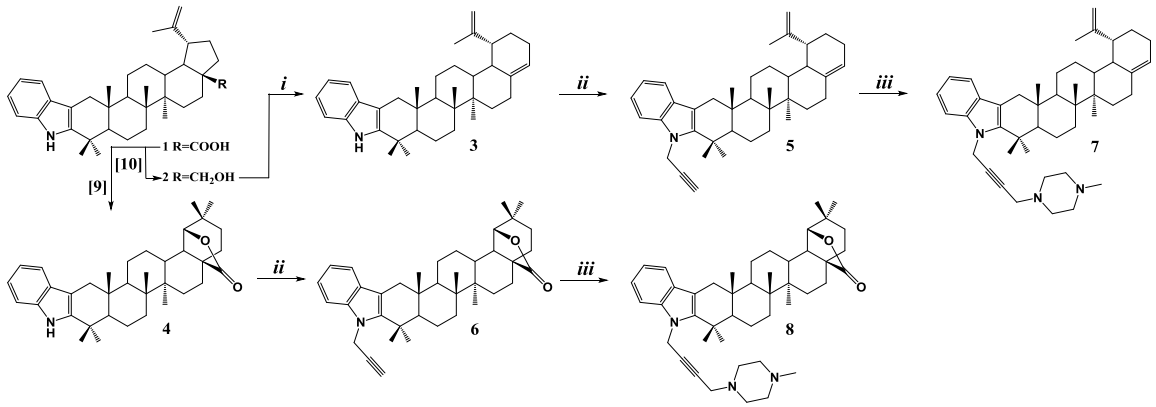
In this work, the first example of *N*-propargylation of indolo-triterpenoids is described and their application for Mannich reaction is presented.

2. Results

At first, the triterpenic indoles were prepared, containing one reactive center for the following propargylation. For this purpose we have modified 2,3-indolo-betulinic acid **1** at the E-ring. The reduction of carboxylic group with $LiAlH_4$ gave 2,3-indolo-betulin **2**, which was dehydrated using phosphorus(V) oxychloride in pyridine to give abeo-derivative **3**. 2,3-Indolo-28-oxo-allobetulone **4** was obtained by Wagner–Meerwein rearrangement of compound **1** in the presence of formic acid (Scheme 1).

Then the reaction of E-ring modified indoles **3** and **4** with propargyl bromide and NaH in DMF provided the *N*-substituted derivatives **5** and **6** with 68 and 70% yields. The structure of compounds **5** and **6** was ascertained by NMR spectroscopy. Thus, the disappearance of signals of amine group protons of indolo-fragment at δ 7.71 and 7.76 ppm was observed. The signals of the acetylene group at δ 72.2–72.3 ppm (^{13}C-NMR) and δ 1.88 and 2.38 ppm (^{1}H-NMR), as well as methylene (δ 5.02–5.04 ppm (^{1}H-NMR)) were characteristic (see Supplementary Materials).

The reaction of *N*-propargylindoles **5** and **6** with *N*-methylpiperazine using Mannich reaction (secondary amine, paraformaldehyde, NaOAc, CuI) gave indolo-*N*-methylpiperazine conjugates **7** and **8** with 72 and 77% yields. The ^{1}H-NMR spectra of Mannich bases **7** and **8** showed typical signals of the *N*-methylpiperazine fragment: the methyl group as a singlet at δ 2.20–2.40 ppm and the methylene groups as a multiplet at δ 2.38–2.78 and 2.44–2.80 ppm. The signals of the acetylene were observed at δ 70.9–81.0 ppm (^{13}C-NMR).



Scheme 1. Synthesis of propargylaminoalkynyl-triterpenoids. *Reagent and conditions*: *i*. $POCl_3$, pyridine, reflux, 8 h; *ii*. Propargyl bromide, NaH, DMF, 0-5 °C, 2 h; *iii*. *N*-methylpiperazine, paraformaldehyde, NaOAc, CuI, 60 °C, 10 h.

3. Materials and Methods

The spectra were recorded at the Center for the Collective Use 'Chemistry' of the Ufa Institute of Chemistry, part of the Ufa Federal Research Centre of the Russian Academy of Sciences. ^{1}H and ^{13}C-NMR spectra were recorded on a "Bruker AM-500" (Bruker, Billerica, MA, USA, 500 and 125.5 MHz respectively, δ, ppm, Hz) in $CDCl_3$, internal standard tetramethylsilane. Mass spectra were obtained on a liquid chromatograph–mass spectrometer LCMS-2010 EV (Shimadzu, Kyoto, Japan). Melting points were detected on a micro table "Rapido PHMK05" (Nagema, Dresden, Germany). Optical rotations were measured on a polarimeter "Perkin-Elmer 241 MC" (PerkinElmer, Waltham, MA, USA) in a tube length of 1 dm. Elemental analysis was performed on a Euro EA-3000 CHNS analyzer (Eurovector, Milan, Italy); the main standard is acetanilide. Thin-layer chromatography analyses were performed on Sorbfil plates (Sorbpolimer, Krasnodar, Russian Federation), using the solvent system chloroform-ethyl acetate, 40:1. Substances were detected by a 10% solution of a sulfuric acid solution with subsequent

heating at 100–120 °C for 2–3 min. Compounds **1** [9] and **4** [9], **2** [11] were obtained according to the methods described previously.

3.1. [3,2b]Indolo-lup-20(29),17(28)-dien (**3**)

A mixture of compound **2** (0.51 g; 1 mmol) and $POCl_3$ (0.09 mL; 1 mmol) in pyridine (15 mL) was refluxed for 8 h, then poured into 50 mL of water and the precipitate was filtered off, and washed with water until neutral pH. The residue was purified by Al_2O_3 column chromatography using petroleum ether as eluent to afford compound **3** as a white crystals (0.42 g, 85%): $[\alpha]_D^{20}$ +7 (c 0.75, CH_2Cl_2), m.p. 207 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 500 MHz): 7.71 (1H, br.s, NH), 7.51−7.10 (4H, m, arom), 5.44 (1H, s, H-28), 4.72 and 4.81 (2H, both s, *J* = 2.0 Hz, H-29), 2.91−2.88 (2H, m, CH_2), 2.31−1.01 (18H, m, CH, CH_2), 1.89 (3H, s, H-30), 1.32, 1.24, 1.15, 1.09, 0.92 (15H, all s, $5CH_3$). ^{13}C-NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 151.0 (C-20), 141.7 (C-17), 140.9 (C-arom), 136.2 (C-arom), 128.4 (C-arom), 120.9 (C-28), 118.9 (C-arom), 118.6 (C-arom), 118.0 (C-arom), 110.4 (C-29), 108.9 (C-arom), 107.1 (C-arom), 55.9, 53.4, 49.4, 46.6, 44.5, 42.4, 40.7, 38.3, 37.4, 36.7, 34.2, 33.9, 33.5, 32.8, 30.9, 27.9, 26.9, 23.9, 22.6, 21.9, 21.4, 19.3, 16.6, 15.8. Anal. Calcd for $C_{36}H_{49}N$: C, 87.21; H, 9.96. Found: C, 87.22; H, 9.97. MS (APCI): *m*/*z* $[M + H]^+$ 497.80, calcd for $C_{36}H_{49}N$: 496.79.

3.2. [3,2b]Indolo-N-prop-2-yn-1-yl-lup-20(29),17(28)-dien (**5**)

NaH (0.028 g; 1.1 mmol), after washing with dry hexane, was suspended in dry DMF (5 mL) followed by the addition of compound **3** (0.50 g; 1 mmol). After the color change of the reaction mixture, propargyl bromide (0.178 g; 1.5 mmol) was added drop-wise. Reaction mixture was stirred for 2 h at 0–5 °C. After the completion of the reaction, ice cooled water (10 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were then washed with water (3 × 50 mL) and dried over $CaCl_2$. Organic phase was concentrated under vacuum and the crude was purified by flesh chromatography (eluent petroleum ether-Et_2O (3:1) to procedure compound **5** as a yellow powder (0.36 g, 68%): $[\alpha]_D^{20}$ +36 (c 0.75, CH_2Cl_2), m.p. 195–197 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 500 MHz): 7.48−7.05 (4H, m, arom), 5.40 (1H, s, H-28), 5.04 (2H, s, CH_2), 4.78−4.70 (2H, both s, *J* = 2.0 Hz, H-29), 2.90−2.84 (2H, m, CH_2), 2.38 (1H, s, C≡CH), 2.23−0.95 (21H, m, CH, CH_2), 1.80 (3H, s, H-30), 1.40, 1.21, 1.13, 1.05, 0.90 (15H, all s, $5CH_3$). ^{13}C-NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 151.0 (C-20), 141.7 (C-17), 140.9 (C-arom), 136.1 (C-arom), 128.3 (C-arom), 120.9 (C-28), 118.9 (C-arom), 118.5 (C-arom), 117.9 (C-arom), 110.4 (C-29), 108.9 (C-arom), 107.1 (C-arom), 80.1 (C-38), 72.2 (C-39), 55.4, 53.3, 49.4, 46.5, 44.4, 42.3, 40.9, 38.3, 37.4, 34.8, 33.9, 32.7, 30.9, 30.4, 29.5, 28.3, 26.8, 23.8, 23.2, 22.5, 21.6, 19.3, 16.6, 15.7, 14.9. Anal. Calcd for $C_{39}H_{51}N$: C, 87.75; H, 9.63. Found: C, 87.72; H, 9.65. MS (APCI): *m*/*z* $[M + H]^+$ 535.82, calcd for $C_{39}H_{51}N$: 534.84.

3.3. [3,2b]Indolo-N-prop-2-yn-1-yl-28-oxo-allobetulon (**6**)

Compound **6** was obtained from compound **4** (0.53 g; 1 mmol) by a procedure similar to the synthesis of compound **5** as a yellow powder (0.40 g, 70%): $[\alpha]_D^{20}$ +80 (c 0.75, CH_2Cl_2), m.p. 156 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 500 MHz): 7.49−7.02 (4H, m, arom), 5.02 (2H, s, CH_2), 4.02 (1H, s, H-19), 2.92−2.82 (2H, m, CH_2), 1.88 (1H, s, C≡CH), 2.23−1.00 (20H, m, CH, CH_2), 1.42, 1.28, 1.18, 1.00, 0.95, 0.88, 0.85 (21H, all s, $7CH_3$). ^{13}C-NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 180.0 (C-28), 140.9 (C-arom), 136.2 (C-arom), 128.3 (C-arom), 121.4 (C-arom), 118.9 (C-arom), 117.9 (C-arom), 110.4 (C-arom), 106.8 (C-arom), 86.1 (C-19), 78.9 (C-38), 72.3 (C-39), 55.6, 53.5, 50.3, 50.1, 46.7, 38.1, 37.5, 36.2, 34.8, 33.1, 32.9, 32.4, 31.9, 30.9, 29.5, 28.8, 28.0, 26.7, 25.6, 24.0, 23.1, 21.6, 21.4, 19.1, 16.8, 15.4, 13.7. Anal. Calcd for $C_{39}H_{51}NO_2$: C, 82.78; H, 9.09; N, 2.48. Found: C, 82.77; H, 9.08; N, 2.48. MS (APCI): *m*/*z* $[M + H]^+$ 567.83, calcd for $C_{39}H_{51}NO_2$: 566.82.

3.4. [3,2b]Indolo-N-(4-(4-methylpiperazin-1-yl)but-2-yn-1-yl)- lup-20(29),17(28)-dien (**7**)

N-methylpiperazine (0.72 mmol; 80 μL), paraformaldehyde (0.18g; 6 mmol), NaOAc (0.25g; 3 mmol) and CuI (6 mg; 0.03 mmol) were added to a solution of compound **5** (0.32 g; 0.6 mmol) in dry

dioxane (12 mL). The reaction mixture was stirred under argon for 10 h at 60 °C. After the reaction was complete by thin-layer chromatography the mixture was diluted with water and extracted with $CHCl_3$ (3 × 20 mL). The combined organic layer was washed with water (3 × 50 mL), dried over $CaCl_2$ and evaporated under reduced pressure. The residue was purified by SiO_2 column chromatography (eluent $CHCl_3$–MeOH 100:0→90:10) with obtaining of compound **7** as a yellow solid (0.28 g, 72%), $[\alpha]_D^{20}$ +97 (c 0.75, CH_2Cl_2), m.p. 188-189 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 500 MHz): 7.48–7.02 (4H, m, arom), 5.38 (1H, s, H-28), 5.02 (2H, s, CH_2), 4.69–4.78 (2H, both s, *J* = 2.0 Hz, H-29), 3.69 (1H, s, H-18), 3.21 (2H, s, CH_2), 2.78–2.38 (8H, m, $4CH_2$), 2.40 (3H, s, NCH_3), 2.28–1.15 (22H, m, CH, CH_2), 1.78 (3H, s, H-30), 1.41, 1.32, 1.18, 1.10, 0.89 (15H, all s, $5CH_3$). ^{13}C-NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 151.0 (C-20), 141.6 (C-17), 140.4 (C-arom), 137.3 (C-arom), 127.9 (C-arom), 121.2 (C-28), 119.1 (C-arom), 118.5 (C-arom), 117.9 (C-arom), 109.2 (C-29), 108.9 (C-arom), 108.1 (C-arom), 81.0 (C-38), 78.5 (C-39), 55.5, 55.3, 54.2, 50.6, 49.5, 46.8, 46.0, 45.4, 44.8, 42.3, 40.9, 39.7, 37.9, 37.7, 35.0, 34.9, 33.8, 33.5, 32.7, 29.6, 28.3, 26.9, 24.2, 23.8, 22.4, 21.9, 21.7, 19.3, 16.4, 15.7, 14.9. Anal. Calcd for $C_{45}H_{63}N_3$: C, 83.67; H, 9.83; N, 6.50. Found: C, 83.65; H, 9.84; N, 6.53. MS (APCI): *m*/*z* $[M + H]^+$ 648.02, calcd for $C_{45}H_{63}N_3$: 647.01.

3.5. [3,2b]Indolo-N-(4-(4-methylpiperazin-1-yl)but-2-yn-1-yl)-28-oxo-allobetulon (**8**)

Compound **8** was obtained from **6** (0.34 g; 0.6 mmol) by a procedure similar to the synthesis of compound **7** as a yellow resin (0.31 g, 77%), $[\alpha]_D^{20}$ +17 (c 0.75, CH_2Cl_2), m.p. 165–167 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 500 MHz): 7.45–7.05 (4H, m, arom), 5.05 (2H, s, CH_2), 4.00 (1H, s, H-19), 3.22 (2H, s, CH_2), 2.80–2.44 (8H, m, $4CH_2$), 2.20 (3H, s, NCH_3), 2.30–1.10 (22H, m, CH, CH_2), 1.45, 1.32, 1.30, 1.09, 1.00, 0.92, 0.88 (21H, all s, $7CH_3$). ^{13}C-NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 179.9 (C-28), 140.4 (C-arom), 137.3 (C-arom), 127.9 (C-arom), 121.3 (C-arom), 119.2 (C-arom), 117.9 (C-arom), 109.2 (C-arom), 107.9 (C-arom), 85.9 (C-19), 78.2 (C-38), 70.9 (C-39), 55.6, 53.9, 50.3, 46.7, 46.2, 41.7, 40.6, 39.9, 38.1, 37.8, 36.2, 35.0, 34.6, 33.6, 33.1, 32.4, 31.9, 29.7, 29.6, 28.8, 27.9, 26.7, 25.6, 23.9, 21.7, 21.5, 19.4, 18.7, 19.1, 17.0, 16.6, 15.4, 13.7. Anal. Calcd for $C_{45}H_{63}N_3O_2$: C, 79.72; H, 9.37; N, 6.20. Found: C, 79.73; H, 9.36; N, 6.22. MS (APCI): *m*/*z* $[M + H]^+$ 680.01, calcd for $C_{45}H_{63}N_3O_2$: 679.02.

4. Conclusions

The first synthesis of triterpenic *N*-propargylindoles and their modification using a Cu(I)-catalyzed Mannich reaction were achieved.

Supplementary Materials: ^{1}H and ^{13}C spectra for compounds are available online.

Author Contributions: E.K. prepared and corrected the manuscript; A.P. and G.B. conducted the experiment, and did structure elucidation and prepared the manuscript; O.K. brought the idea, and managed the research and prepared the manuscript.

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

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