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Click chemistry decoration of amino sterols as promising strategy to developed new leishmanicidal drugs

Exequiel O.J. Porta [a], Paulo B. Carvalho [b], Mitchell A. Avery [c], Babu L. Tekwani [d], Guillermo R. Labadie [a,*]

[a] Instituto de Química Rosario (IQUIR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina
[b] School of Pharmacy, Notre Dame of Maryland University, Baltimore, MD 21210, USA
[c] Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, MS 38677, USA
[d] National Center for Natural Products Research & Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677, USA

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ABSTRACT

A series of 1,2,3-triazolylsterols was prepared from pregnenolone through reductive amination and copper(I)-catalyzed azide-alkyne cycloaddition (click chemistry). The newly generated stereocenter of the key propargylamino intermediate provided a mixture of diastereomers which were separated chromatographically, and the configuration of the *R* isomer was determined by X-ray crystallography. Ten triazolyl sterols were prepared, and the products and intermediates were screened *in vitro* against different parasites, with some compounds presenting IC_{50} values in the low micromolar range against *Leishmania donovani*.

1. Introduction

Parasitic diseases have burdened the world since the early days of mankind and several herbal and mineral extracts were used to treat such maladies until the 19th century. The development of new chemical processes allowed for the isolation of natural compounds and preparation of chemical entities displaying antiparasitic properties, culminating with the introduction of a great number of new drugs in the middle of the 20th century to treat many infectious diseases, including malaria and leishmaniasis [1]. However, the pace with which new drugs were introduced in the market was not kept over the rest of the century, especially in poorer countries, where diseases like malaria, trypanosomiasis and leishmaniasis are still responsible for millions of deaths [2–5]. Current treatments present many disadvantages, such as undesirable side effects and development of resistant parasite strains, creating an urgent need for new drugs [6].

Among many natural substances potentially useful for the development of new antiparasitic drugs, sterols are an important and ubiquitous class of compounds, constantly isolated from new natural sources and modified synthetically on the polycyclic system and side chain [7–9]. These modifications modulate their interactions with molecular targets, producing a wide spectrum of biological activities, and generating compounds showing antibacterial [10], antitubercular [11], and antiprotozoal [12] activities. Parasites, like any other organism, require sterols for survival, and are capable of salvaging their host's sterols to survive if their sterol biosynthetic pathways are inhibited [13]. Fungi and protozoa, such as trypanosomatids, produce and use ergosterol, in contrast to the mammalian cells' use of cholesterol. Ergosterol is biosynthesized by a sequence of enzymes that diverge in some points from the mammals' counterpart, offering a convenient target for new antifungal and antiparasitic agents [14]. Sterols isolated from natural sources have also shown activity against *Leishmania* sp. [15] and *Plasmodium* sp. [16]. Demethylase *Erg11* and methyl transferase *Erg 6* have been targeted by sterol derivatives with heteroatoms or heterocycles on the side chain, and some of these modified sterols have exhibited activity against *Leishmania* spp. and *Trypanosoma cruzi* [17–19].

The design of strategies to explore the chemical space through chemical diversity and construction of new chemical libraries are key steps on the search for new active compounds, especially when the specific target is unknown [20]. When the search starts with a validated target the best strategy is to prepare focused libraries [21], an approach that has been greatly facilitated by the application of click chemistry in medicinal chemistry [22,23], which has been commonly associated to the preparation of 1,2,3-triazoles through Cu(I) catalysis [24]. Due to the simplicity of this reaction,

* Corresponding author. Tel.: +54 341 4370477x108; fax: +54 341 4370477x112.
E-mail address: labadie@iquir-conicet.gov.ar (G.R. Labadie).

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libraries of 1,2,3-triazoles have been prepared as selective enzyme inhibitors [25–27], and against parasites [27–30], tuberculosis [31,32], and cancer [33,34]. Recently, this versatile reaction has been used to make libraries of heterocyclic steroids [34–36].

In search of new chemical entities (NCE) with antiparasitic properties, we designed a simple strategy to prepare new heterocyclic steroids. Based on the literature, we hypothesized a synergistic effect of nitrogen on the lateral chain along with a heterocyclic ring, improving biological activity. To test this hypothesis, we introduced a propargylamine unit on the side chain of pregnenolone through reductive amination, which is both the source of the amino group and of the scaffold to build heterocycles through click chemistry (Fig. 1). The compounds prepared were assayed for antiparasitic activity and cytotoxicity.

2. Experimental section

2.1. General

Chemical reagents were purchased from commercial suppliers and used without further purification, unless otherwise noted. Solvents (hexanes, ethyl acetate, CH_2Cl_2, Et_2O) were distilled prior to use. CH_2Cl_2 was dried over P_2O_5. DMF was distilled from BaO. Reactions were monitored on precoated silica gel G or GP TLC plates. Spots were visualized under 254 nm UV light and/or by TLC staining [37]. All reactions were performed under an atmosphere of nitrogen using oven-dried glassware and standard syringe/septa techniques. Column chromatography was performed with silica gel 60 (230–400 mesh). Yields were calculated for material judged homogeneous by thin layer chromatography (TLC) and nuclear magnetic resonance (^{1}H NMR).

2.2. Characterization of the products

^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance II 300 MHz (75.13 MHz) using $CDCl_3$ as solvent. Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane as internal standard and coupling constants are in hertz (Hz). Assignment of proton resonances was confirmed by correlated spectroscopy. High-resolution mass spectra (ESI-HRMS) were recorded on a Micromass spectrometer with lock spray source or on a Bruker MicroTOF II. IR spectra were obtained using an FT-IR Shimadzu spectrometer and only partial spectral data are listed. Melting points were measured on an Electrothermal 9100 apparatus and are uncorrected.

2.3. Chemical synthesis

2.3.1. Synthesis of propargylamino intermediate **1** by reductive amination of pregnenolone

To a solution of pregnenolone (1.0 g, 3.16 mmol) in 20 mL of THF, propargylamine (1.05 g, 19 mmol), $NaBH(AcO)_3$ (1.34 g, 6. 32 mmol) and finally 4 Å molecular sieves (100 mg) were added in this order and the reaction mixture was stirred at room temperature. Additional molecular sieves were added every 48 h until the reaction was completed in 6 days. Then, the reaction was quenched by addition of 5% $NaHCO_3$ (50 mL) and the layers were separated and filtered to remove the molecular sieves. The aqueous phase was extracted with ether (4 × 20 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated. The products were purified by column chromatography in silica gel with increasing ethyl acetate/hexane gradient to yield a less polar fraction composed by **1*R***, 552 mg, 49%, and a more polar fraction containing **1*S***, 544 mg, 48%.

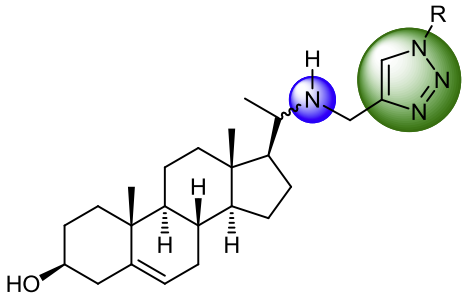


Fig. 1. Side chain functionalized steryl analogues designed.

*2.3.1.1. (20R)-20-(prop-2-yn-1-ylamino)pregn-5-en-3β-ol (**1R**).* Light yellow solid (552 mg, yield 49%, reaction time 6d). **Mp:** 148–149 °C **IR (KBr):** υ_{max} (cm^{-1}) = 3475, 3363, 3279, 2936, 2882, 2361, 1729, 1450, 1376. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.33 ppm (d, 1H, *J* = 5.2 Hz, C6–**H**); 3.50 (m, 1H, C3–**H**); 3.48, 3.35 (dd, 2H, *J* = 17.2 Hz, 2.4 Hz, –NH–C**H$_2$**–); 2.84 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–**H**); 2.18 (t, 1H, *J* = 2.4 Hz, —C≡C**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.30 (m, 1H, C17–**H**); 1.06 (m, 1H, C14–**H**); 1.00 (s, 3H, C19–**H**); 0.98 (m, 1H, C9–**H**); 0.96 (m, 3H, *J* = 6.0 Hz, C21–**H**) and 0.76 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 140.8 ppm (**C5**); 121.5 (**C6**); 82.5 (—**C**≡CH); 71.7 (**C3**); 71.0 (—C≡**C**H); 56.4 (**C14**); 56.1 (**C17**); 53.8 (**C20**); 49.9 (**C9**); 42.3 (**C4**); 42.0 (**C13**); 40.2 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 34.9 (NH—CH_2—CH≡); 31.8 (**C2**); 31.8 (**C8**); 31.6 (**C7**); 26.7 (**C15**); 24.1 (**C16**); 21.1 (**C11**); 19.4 (**C19**); 18.4 (**C21**) and 12.3 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{24}H_{37}NO$ $(M+H)^+$, 356.2953; found, 356.2953.

*2.3.1.2. (20S)-20-(prop-2-yn-1-ylamino)pregn-5-en-3β-ol (**1S**).* Light yellow solid (544 mg, yield 48%, reaction time 6d). **Mp** 145–146 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3283, 3268, 2894, 2885, 1646, 1443, 1421, 1375, 1382, 1249, 1097, 1070, 718. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.35 ppm (d, 1H, *J* = 4.7 Hz, C6–**H**); 3.52 (m, 1H, C3–**H**); 3.50, 3.34 (dd, 2H, *J* = 17.2 Hz, 2.4 Hz, –NH–C**H$_2$**–); 2.74 (m, 1H, C20–**H**); 2.27 (d, 2H; *J* = 7.5 Hz, C4–**H**), 2.19 (t, 1H, *J* = 2.4 Hz, —C≡C**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.06 (m, 1H, C14–**H**); 1.06 (d, 3H, *J* = 6.1 Hz, C21–**H**); 1.06 (s, 3H, C19–**H**) 1.02 (m, 1H, C9–**H**) and 0.72 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 140.8 ppm (**C5**); 121.6 (**C6**); 82.6 (—NH—CH_2—**C**≡); 71.8 (**C3**); 71.0 (—**C**≡CH); 56.6 (**C17**); 56.3 (**C14**); 54.6 (**C20**); 50.0 (**C9**); 42.3 (**C4**); 42.1 (**C13**); 39.3 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 35.1 (—NH—**C**H_2—C≡); 31.8 (**C8**); 31.7 (**C7**); 31.6 (**C2**); 26.9 (**C15**); 24.2 (**C16**); 20.9 (**C11**); 19.4 (**C19**); 18.6 (**C21**) and 12.3 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{24}H_{37}NO$ $(M+H)^+$, 356.2953; found, 356.2968.

2.3.2. General procedure for the Cu(I) mediated 1,3-dipolar cycloaddition

Alkyne (1 eq) and the azide (1.1 eq) were suspended in 10 mL/eq of ^{t}BuOH:H_2O (1:1) and then 1 M $CuSO_4$ solution and finally 1 M sodium ascorbate solution were added and the mixture stirred overnight at room temperature. Brine was added and the solution was extracted with dichloromethane. Combined organic extracts were dried over sodium sulfate and evaporated. Products were purified by column chromatography in silica gel with increasing ethyl acetate/methanol gradients.

*2.3.2.1. (20R)-20-(((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amino)-pregn-5-en-3β-ol (**2A-R**).* Light yellow solid (47 mg, yield 89%,

reaction time 12 h). **Mp** 130–131 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3357, 2937, 2833, 1663, 1497, 1456, 1383. **^{1}H NMR** ($CDCl_3$): δ = 7.35 ppm (s, 1H **C5 triazole-H**); 7.35 (m, 3**H** meta and para- aromatic); 7.25 (m, 2**H** ortho-aromatic); 5.50 (s, 2H, –C**H$_2$**–phenyl); 5.33 (d, 1H, *J* = 5.2 Hz, C6–**H**); 3.97, 3.75 (d, 2H, *J* = 13,7 Hz, C22–**H$_2$**); 3.52 (m, 1H, C3–**H**); 2.62 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.06 (m, 1H, C14–**H**); 1.02 (d, 3H, *J* = 6.1 Hz, C21–**H**); 1.00 (s, 3H, C19–**H**); 0.98 (m, 1H, C9–**H**); and 0.60 (s, 3H, C18–**H**). **^{13}C NMR** ($CDCl_3$): δ = 140.8 ppm (**C5**); 134.8 (**C4 triazole**); 129.1 (ipso-**C aromatic**); 128.7 (p and m-**C aromatic**); 128.0 (o-**C aromatic**); 121.5 (**C6**); 121.5 (**C5 triazole**); 71.7 (**C3**); 56.4 (**C14**); 56.1 (**C17**); 55.1 (**C20**); 54.1 (–**C**H$_2$–phenyl); 50.0 (**C9**); 42.3 (**C4**); 42.1 (**C13**); 41.7 (NH–**C**H$_2$-triazole); 40.0 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 31.8 (**C2**); 31.8 (**C7**); 31.7 (**C8**); 26.7 (**C15**); 24.1 (**C16**); 21.1 (**C11**); 19.4 (**C19**); 18.9 (**C21**) and 12.2 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{24}H_{37}NO$ $(M+H)^+$, 489.3593; found, 489.3593.

*2.3.2.2. Ethyl 2-(4-((((20R)-1-((20-pregn-5-en-3β-yl)ethyl)amino)-methyl)-1H-1,2,3-triazol-1-yl)acetate (**2B-R**).* Light orange oil (42 mg, yield 78%, reaction time 12 h). **IR (KBr):** υ_{max} (cm^{-1}) = 3435, 2940, 1750. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.59 ppm (s, C5-**H triazole**); 5.32 (d, 1H, *J* = 5.2 Hz, C6–**H**); 5.13 (d, 2H, *J* = 1.35 Hz, triazole-N1-C**H$_2$**–COOEt); 4.25 (q, 2H, *J* = 7.1 Hz, –O–C**H$_2$**–); 3.91, 3.64 (d, 2H, *J* = 13,8 Hz, C22–**H$_2$**); 3.51 (m, 1H, C3–**H**); 2.67 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.29 (t, 3H, *J* = 7.2 Hz, –O–CH$_2$–C**H$_3$**); 1.06 (m, 1H, C14–**H**); 1.05 (d, 3H, *J* = 6.1 Hz, C21–**H**); 0.98 (s, 3H, C19–**H**); 0.98 (m, 1H, C9–**H**); and 0.65 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 166.3 ppm (**C**=O); 147.4 (C23, **C4 triazole**); 140.8 (**C5**); 123.0 (**CH triazole**); 121.5 (**C6**); 71.7 (**C3**); 63.4 (–O–**C**H$_2$–CH$_3$); 56.4 (**C14**); 56.1 (**C17**); 55.1 (**C20**); 50.9 (triazole N1–**C**H$_2$–CO); 50.0 (**C9**); 42.3 (**C4**); 42.1 (**C13**); 41.6 (N–**C**H$_2$-triazole); 40.0 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 31.8 (**C2**); 31.8 (**C7**); 31.6 (**C8**); 26.8 (**C15**); 24.2 (**C16**); 21.1 (**C11**); 19.4 (**C19**); 18.9 (**C21**); 14.1 (O–CH$_2$–**C**H$_3$) and 12.2 (**C18**). **HRMS** (**ESI**): *m/z* calcd. for $C_{28}H_{45}N4O_3$ $(M+H)^+$, 485.3491; found, 485.3492.

*2.3.2.3. (20R)-20-(((1-octyl-1H-1,2,3-triazol-4-yl)methyl)amino)-pregn-5-en-3β-ol (**2C-R**).* Light yellow solid (48 mg, yield 83%, reaction time 12 h). **Mp** 125–127 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3432, 2927, 2856, 1615, 1456, 1149, 1113, 1108, 1085, 1057, 1024. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.43 ppm (s, C5 triazole-**H**); 5.32 (d, 1H, *J* = 5,2 Hz, C6–**H**); 4.31 (t, 2H, *J* = 6.9 Hz, N1 triazole-C**H$_2$**-Octyl); 3.98, 3.78 (d, 2H, *J* = 13,5 Hz, C22–**H$_2$**); 3.51 (m, 1H, C3–**H**); 2.65 (m, 1H, C20–**H**); 2.37; (m, 2H, N1 triazole-CH$_2$–C**H$_2$**–CH$_2$); 2.26 (m, 2H; C4–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.51 (m, 2H, N1 triazole-CH$_2$–CH$_2$–C**H$_2$**–CH$_2$–); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.29 to 1.24 (m, 8 H, –C**H$_2$**– octyl); 1.23 (d, 3H, C21–**H**); 1.06 (m, 1H, C14–**H**); 0.98 (s, 3H, C19–**H**); 0.98 (m, 1H, C9–**H**); 0.86 (t, 3H, *J* = 6,7 Hz, Octyl–C**H$_3$**) and 0.64 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 140.8 ppm (**C4 triazole**); 140.8 (**C5**); 122.5 (**C5 triazole**); 121.5 (**C6**); 71.7 (**C3**); 56.4 (**C14**); 50.3 (N1 triazole-**C**H$_2$–); 50.0 (**C9**); 42.3 (**C4**); 42.1 (**C13**); 40.8 (NH–**C**H$_2$-triazole); 40.0 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 31.8 (**C2**); 31.7 (**C7**); 31.6 (**C8**); 30.3 (N1 triazole-CH$_2$–**C**H$_2$–); 29.1 (**C30**); 28.9 (**C28**; CH$_2$); 26.5 (**C15**); 24.2 (**C16**); 22.6 (**C27**, CH$_2$); 22.6 (**C31**, CH$_2$); 21.1 (**C11**); 19.4 (**C19**); 18.9 (**C21**); 14.1 (**C28**, CH$_3$) and 12.2 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{32}H_{55}N_4O$ $(M+H)^+$, 511.4370; found, 511.4375.

*2.3.2.4. (20R)-20-(((1-((E/Z)-3,7-dimethylocta-2,6-dienyl)-1H-1,2,3-triazol-4-yl)methyl)amino)pregn-5-en-3β-ol (**2D-R**).* Light yellow oil (57 mg, yield 90%, reaction time 12 h). **IR (KBr):** υ_{max} (cm^{-1}) = 3311, 2954, 2939, 2861, 1868, 1451, 1377, 1136, 1051, 800. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.42 ppm (s, C23′–**H**); 7.41 ppm (s, C23–**H**); 5.40 (t, 1H, *J* = 7.3 Hz, C26–**H**, C26′–**H**); 5.32 ppm (d, 1H, *J* = 4.8 Hz, C6–**H**); 5.05 (m, 1H, C31–**H**, 31′–H); 4.92 (t, 2H, *J* = 6,72 Hz, C25–**H**, C25′–**H**); 3.98, 3.75 (d, 2H, *J* = 13,6 Hz, C22–**H**); 3.50 (hept, 1H, *J* = 4,2 Hz, C3–**H**); 2.64 (m, 1H, C20–**H**); 2.27 (m, 2H; C4–**H**); 2.10 (m, 4H, C29,30,29′,30′–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.79 (s, 1H, C28′–**H**); 1.76 (s, C28–**H**); 1.67 (s, C34–**H**, C34′–**H**); 1.60 (s, C33–**H**, C33'–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.06 (d, 3H, *J* = 6.1 Hz, C21–**H**);1.06 (m, 1H, C14–**H**); 1.02 (m, 1H, C9–**H**); 0.98 (s, 3H, C19–**H**) and 0.64 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 146.8 (**C23, C23′**); 143.1 ppm (**C27′**); 142.9 (**C27**); 140.8 ppm (**C5**); 132.7 (**C32′**); 132.1 (**C32**); 123.4 (**C31**); 123.2 (**C31′**); 121.5 (**C6**); 121.5 (**C24**, **C24′**); 118.0 (**C26′**); 117.1 (**C26**); 71.7 (**C3**); 56.4 (**C14**); 56.1 (**C17**); 55.2 (**C20**); 50.0 (**C9**); 47.8 (**C25**); 47.7 (**C25′**); 42.3 (**C4**); 42.1 (**C13**); 41.7 (**C22**); 40.0 (**C29**); 39.4 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 32.1 (**C29′**); 31.8 (**C8**); 31.8 (**C7**); 31.6 (**C2**); 26.7 (**C30**); 26.3 (**C30′**); 25.7 (**C34**, **C34′**); 24.1 (**C16**); 23.4 (**C28′**); 21.1 (**C11**); 19.4 (**C19**); 18.9 (**C21**); 17.7 (**C33**, **C33′**); 16.5 (**C28**) and 12.2 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{34}H_{55}N_4O$ $(M+H)^+$, 535.4370; found, 535.4376.

*2.3.2.5. (20R)-20-(((1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl)methyl)amino)pregn-5-en-3β-ol (**2E-R**).* Light yellow solid (51 mg, yield 94%, reaction time 12 h). **Mp** 149–150 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3419, 3384, 3315, 2862, 2852, 2836, 1464, 1451, 1443, 1069, 1056. 1049, 841, 730, 694. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.44 ppm (s, 1H, C5 triazole-**H**); 7.28 (m, 2H, m-aromatic,); 7.22 (m, 1H p-aromatic); 7.17 (m, 2H o-aromatic); 5.31 (d, 1H, *J* = 5.0 Hz, C6–**H**); 4.32 (t, 2H, *J* = 7,0 Hz, N1 triazole-C**H$_2$**–); 3.99, 3.76 (d, 2H, *J* = 13,7 Hz, C 22–**H$_2$**); 3.51 (m, 1H, C3–**H**); 2.63 (t, 2H, *J* = 7.5 Hz, –C**H$_2$**–phenyl); 2.62 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–**H**); 2.23 (m, 2H, C26–H); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.06 (m, 1H, C14–**H**); 1.06 (d, 3H, *J* = 6.2 Hz, C21–**H**); 0.98 (m, 1H, C9–**H**); 0.97 (s, 3H, C19–**H**) and 0.64 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 146.7 ppm (**C4 triazole**); 140.9 (**C5**); 140.2 (**C** ipso-aromatic); 128.6 (**C** m-aromatic); 128.4 (**C** o-aromatic); 126.3 (**C** p-aromatic); 121.7 (**C6**); 121.4 (**C5 triazole**); 71.6 (**C3**); 56.4 (**C14**); 56.0 (**C17**); 55.2 (**C20**); 50.0 (**C9**); 49.4 (–**C**H$_2$–phenyl); 42.3 (**C4**); 42.1 (**C13**); 41.6 (NH–**C**H$_2$-triazole); 40.0 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 32.5 (N1 triazole-**C**H$_2$–); 31.8 (**C2**); 31.8 (**C7**); 31.7 (**C8**); 31.6 (–**C**H$_2$–CH$_2$–Phenyl); 26.7 (**C15**); 24.1 (**C16**); 21.1 (**C11**); 19.4 (**C19**); 18.8 (**C21**) and 12.3 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{33}H_{49}N_4O$ $(M+H)^+$, 517.3901; found, 517.3906.

*2.3.2.6. (20S)-20-(((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amino)-pregn-5-en-3β-ol (**2A-S**).* Light yellow solid (49 mg, yield 76%, reaction time 12 h). **Mp** 130 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3404, 3323, 2930, 2854, 1606, 1456, 1051. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.42 ppm (s, 1H triazol, C5 triazole-**H**); 7.35 (m, 3H, ortho and para aromatic protons); 7.26 (m, 2H, m-aromatic); 5.50 (s, 2H, –C**H$_2$**–phenyl); 5.34 (d, 1H, *J* = 5.2 Hz, C6–**H**); 3.98, 3.78 (d, 2H, *J* = 13,6 Hz, C 22–**H$_2$**); 3.51 (m, 1H, C3–**H**); 2.59 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H,

C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.49 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.30 (m, 1H, C17–**H**); 1.15 (d, 3H, *J* = 6.1 Hz, C21–**H**); 1.06 (m, 1H, C14–**H**); 1.00 (s, 3H, C19–**H**); 1.00 (m, 1H, C9–**H**) and 0.64 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 146.8 ppm (**C4** triazole); 140.8 (**C5**); 134.7 (**C26**, **C** ipso-aromatic); 129.1 (**C29**, C p-aromatic); 128.7 (**C28** and **C30**, C m-aromatic) 128.0 (**C27** and **C31**, C o-aromatic); 121.9 (**C6**); 121.5 (**C5** triazole); 71.6 (**C3**); 56.6 (**C14**); 56.0 (**C17**); 55.9 (**C20**); 54.1 (–**C**H_2–phenyl); 50.0 (**C9**); 42.3 (**C4**); 42.0 (**C13**); 41.3 (**C22**); 39.3 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 31.8 (**C2**); 31.7 (**C7**); 31.6 (**C8**); 27.1 (**C15**); 24.1 (**C16**); 20.9 (**C11**); 19.4 (**C19**); 18.8 (**C21**) and 12.2 (**C18**) **HRMS (ESI):** *m/z* calcd. for $C_{24}H_{37}NO$ $(M+H)^+$, 489.3593; found, 489.3587.

*2.3.2.7. Ethyl 2-(4-((((20S)-1-((20-pregn-5-en-3β-yl)ethyl)amino)-methyl)-1H-1,2,3-triazol-1-yl)acetate (**2B-S**).* Light orange oil (49 mg, yield 78%, reaction time 12 h). **IR (KBr):** υ_{max} (cm^{-1}) = 3446, 2924, 2851, 1749, 1632, 1465, 1384. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.68 ppm (s, **C4** triazole-**H**); 5.33 (d, 1H, *J* = 5.2 Hz, C6–**H**); 5.13 (s, 2H, –C**H$_2$**–CO–); 4.26 (q, 2H, *J* = 7.1 Hz, O–C**H$_2$**–CH_3); 4.07, 3.86 (d, 2H, *J* = 13,8 Hz, C 22–**H$_2$**); 3.52 (m, 1H, C3–**H**); 2.67 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.30 (t, 3H, *J* = 7.1 Hz O –CH_2–C**H$_3$**); 1.19 (d, 3H, *J* = 6.2 Hz, C21–**H**); 1.06 (m, 1H, C14–**H**); 1.00 (s, 3H, C19–**H**); 0.98 (m, 1H, C9–**H**); and 0.66 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 166.3 ppm (**C**O); 146.6 (**C4** triazole); 140.8 (**C5**); 123.5 (**C5** triazole); 121.5 (**C6**); 71.6 (**C3**); 62.4 (O–**C**H_2–CH_3); 56.6 (**C14**); 55.9 (**C17**); 55.9 (**C20**); 50.9 (–**C**H_2–CO); 50.0 (**C9**); 42.3 (**C4**); 42.0 (**C13**); 41.1 (–NH–**C**H_2–); 39.3 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 31.8 (**C2**); 31.7 (**C7**); 31.6 (**C8**); 27.1 (**C15**); 24.2 (**C16**); 20.9 (**C11**); 19.4 (**C19**); 18.6 (**C21**); 14.1 (O –CH_2–**C**H_3) and 12.2 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{28}H_{45}N4O_3$ $(M+H)^+$, 485.3491; found, 485.3475.

*2.3.2.8. (20S)-20-(((1-octyl-1H-1,2,3-triazol-4-yl)methyl)amino)pregn-5-en-3β-ol (**2C-S**).* Light yellow solid (42 mg, yield 75%, reaction time 12 h). **Mp** 116–117 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3431, 2927, 2856, 1615, 1456, 1149, 1133, 1108, 1085, 1057, 1024. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.61 ppm (s, 1H, C24 triazole-**H**); 5.32 (d, 1H, *J* = 5.2 Hz, C6–**H**); 4.32 (t, 2H, *J* = 7.2 Hz, N1 triazole-C25–**H**); 4.09, 3.88 (d, 2H, *J* = 13,5 Hz, C22–**H$_2$**); 3.51 (m, 1H, C3–**H**); 2.68 (m, 1H, C20–**H**); 2.37 (m, 2H, C26–**H**); 2.26 (m, 2H; C4–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.51 (m, 2H, C27–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.29 (t, 3H, *J* = 7.2 Hz, C28–**H**); 1.24 (m, 8 H, Octyl –C**H$_2$**–); 1.23 (d, 3H, C21–**H**); 1.06 (m, 1H, C14–**H**); 0.99 (s, 3H, C19–**H**); 0.98 (m, 1H, C9–**H**); 0.86 (t, 3H, *J* = 6.6 Hz, C32–**H**) and 0.65 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 140.8 ppm (**C4** triazole); 140.8 (**C5**); 122.5 (**C5** triazole); 121.4 (**C6**); 71.6 (**C3**); 56.6 (**C14**); 56.0 (**C17**); 55.6 (**C20**); 50.4 (N1 triazole-**C**H_2-heptane); 49.9 (**C9**); 42.3 (**C4**); 42.1 (**C13**); 40.8 (–NH–**C**H_2-triazole); 39.2 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 31.8 (**C2**); 31.7 (**C7**); 31.6 (**C8**); 30.3 (N1 triazole-CH_2–**C**H_2-hexane); 29.0 (**C30**; CH_2); 28.9 (**C28**; CH_2); 27.2 (**C29**; CH_2); 26.5 (**C15**); 24.2 (**C16**); 22.6 (**C27**, CH_2); 22.6 (**C31**, CH_2); 20.9 (**C11**); 19.4 (**C19**); 18.3 (**C21**); 14.0 (**C28**, CH_3) and 12.1 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{32}H_{55}N_4O$ $(M+H)^+$, 511.4370; found, 511.4370.

*2.3.2.9. (20S)-20-(((1-((E/Z)-3,7-dimethylocta-2,6-dienyl)-1H-1,2,3-triazol-4-yl)methyl)amino)pregn-5-en-3β-ol (**2D-S**).* Light yellow oil (63 mg, yield 94%, reaction time 12 h). **IR (KBr):** υ_{max} (cm^{-1}) = 3266, 2894, 2884, 1646, 1444, 1375, 1302, 1249, 1097, 1070, 717. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.48 ppm (s, 1H, C23′–**H**); 7.47 ppm (s, 1H, C23–**H**); 5.41 (t, 1H, *J* = 7.3 Hz, C26–**H**, C26′–**H**); 5.33 (d, 1H, *J* = 4.8 Hz, C6–**H**); 5.06 (m, 1H, C31–**H**, C31′–**H**); 4.93 (t, 2H, *J* = 6.7 Hz, C25–**H**, C25′–**H**); 4.00, 3.81 (d, 2H, *J* = 13.4 Hz, C22–**H**); 3.51 (hept, 1H, *J* = 4.2 Hz, C3–**H**); 2.64 (m, 1H, C20–**H**); 2.27 (m, 2H; C4–**H**); 2.10 (m, 4H, C29,30,29′,30′–**H**); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.80 (s, 1H, C28–**H**); 1.77 (s, C28′–**H**); 1.68 (s, C34–**H**,34′–**H**); 1.60 (s, C33–**H**); 1.59 (s, C33′–**H**); 1.58, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.31 (m, 1H, C17–**H**); 1.19 (d, 3H, *J* = 6.1 Hz, C21–**H**); 1.06 (m, 1H, C14–**H**); 1.00 (s, 3H, C19–**H**); 0.99 (m, 1H, C9–**H**) and 0.67 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 146.2 (**C23**, **C23′**) ppm; 143.1 (**C27**); 142.9 (**C27′**); 140.8 (**C5**); 132.6 (**C32**); 132.1 (**C32′**); 123.4 (**C31**); 123.2 (**C31′**); 121.5 (**C6**); 121.3 (**C24**, **C24′**); 117.9 (**C26′**); 117.0 (**C26**); 71.6 (**C3**); 56.6 (**C14**); 56.0 (**C17**); 50.0 (**C9**); 47.9 (**C25**); 47.7 (**C25′**); 42.3 (**C4**); 42.0 (**C13**); 41.4 (**C22**); 39.4 (**C29**); 39.3 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 32.1 (**C29′**); 31.8 (**C8**); 31.7 (**C7**); 31.6 (**C2**); 27.1 (**C15**); 26.3 (**C30**); 26.1 (**C30′**); 25.7 (**C34**, **C34′**); 24.2 (**C16**); 23.4 (**C28′**); 20.9 (**C11**); 19.4 (**C19**); 18.7 (**C21**); 17.7 (**C33**, **C33′**); 16.5 (**C28**) and 12.2 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{34}H_{55}N_4O$ $(M+H)^+$, 535.4370; found, 535.4376.

*2.3.2.10. (20S)-20-(((1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl)methyl) amino)pregn-5-en-3β-ol (**2E-S**).* Light yellow solid (59 mg, yield 90%, reaction time 12 h). **Mp** 118–119 °C. **IR (KBr):** υ_{max} (cm^{-1}) = 3419, 3384, 3314, 2862, 2852, 2836, 1464, 1451, 1443, 1070, 1056, 1049, 841, 730, 694. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.53 ppm (s, 1H, C24 triazole–**H**); 7.28 (m, 2H, m-C–**H**); 7.22 (m, 1H, p-C–**H**); 7.17 (m, 2H, o-C–**H**); 5.34 (d, 1H, *J* = 5.0 Hz, C6–**H**); 4.34 (t, 2H, *J* = 7.0 Hz, N1 triazole-C**H$_2$**–CH_2); 4.05, 3.84 (d, 2H, *J* = 13.7 Hz, C22–**H$_2$**); 3.52 (m, 1H, C3–H); 2.65 (t, 2H, *J* = 7.2 Hz, Ar–C**H$_2$**–); 2.62 (m, 1H, C20–**H**); 2.26 (m, 2H; C4–H); 2.25 (q, 2H, *J* = 7.2 Hz, N1 triazole-CH_2–C**H$_2$**–); 1.98 (m, 2H, C12–**H**); 1.97, 1.55 (m, 2H, C2–**H**); 1.84, 1.10 (m, 2H, C1–**H**); 1.82 (m, 2H, C7–**H**); 1.80, 1.30 (m, 2H, C15–**H**); 1.60, 1.05 (m, 2H, C16–**H**); 1.50 (m, 2H, C11–**H**); 1.43 (m, 1H, C8–**H**); 1.32 (m, 1H, C17–**H**); 1.06 (m, 1H, C14–**H**); 1.06 (d, 3H, *J* = 6.2 Hz, C21–**H**); 1.00 (m, 1H, C9–**H**); 1.00 (s, 3H, C19–**H**) and 0.67 (s, 3H, C18–**H**). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 140.7 ppm (**C4 triazole**); 140.1 (**C5**); 140.1 (**C** ipso-aromatic); 128.6 (**C** m-aromatic); 128.4 (**C** o-aromatic); 126.4 (**C** p-aromatic); 121.6 (**C6**); 121.5 (**C5 triazole**); 71.7 (**C3**); 56.6 (**C14**); 56.0 (**C17**); 56.0 (**C20**); 50.0 (**C9**); 49.5 (–**C**H_2-phenyl); 42.3 (**C4**); 42.2 (**C13**); 42.2 (NH–**C**H_2-triazole); 39.3 (**C12**); 37.2 (**C1**); 36.5 (**C10**); 32.5 (N1 triazole-**C**H_2–); 31.8 (**C2**); 31.8 (**C7**); 31.6 (–**C**H_2–CH_2-phenyl); 27.2 (**C15**); 24.2 (**C16**); 20.9 (**C11**); 19.4 (**C19**) and 12.2 (**C18**). **HRMS (ESI):** *m/z* calcd. for $C_{33}H_{49}N_4O$ $(M+H)^+$, 517.3901; found, 517.3920.

2.4. X-ray

A single crystal X-ray diffraction study was conducted for Compound **1*R***. Colourless crystals were obtained from slow evaporation of a solution in hexanes/ethyl acetate 90:10. A single crystal, approximate dimensions 0.20 × 0.15 × 0.09 mm, was used for data collection on a Bruker Smart Apex II system, using CuKα radiation with a graphite monochromator, fine-focus sealed tube. The crystal was kept at 100 K under a stream of cooled nitrogen gas from a KRYO-FLEX low temperature device. Compound **1*R***, $C_{24}H_{37}NO$, M_r = 355.55, crystallizes with one molecule in the asymmetric unit, monoclinic space group $P2_1$, *a* = 11.7666 (2) Å, *b* = 6.0219 (1) Å, *c* = 14.7197 (2) Å, b = 105.707 (1) Å, *V* = 1004.05 (3) $Å^{3,}$ and *Z* = 2. Data collection, indexing and initial cell refinements were all carried out using APEX II software. Frame integration and final cell refinements were done using SAINT software. The final cell parameters were determined from least-squares refinement on 9876

reflections, with R value = 0.036, $wR(F^2)$ = 0.095. Structure solution, refinement, graphics and generation of publication materials were performed by using SHELXTL, V6.12 software. Hydrogen atoms were placed their expected chemical positions using the HFIX command and were included in the final cycles of least squares with isotropic U_{ij}'s related to the atom's ridden upon. The supplementary crystallographic data can be obtained free of charge from The Cambridge Crystallographic Data Centre, reference number CCDC 819197, via www.ccdc.cam.ac.uk/data_request/cif.

2.5. Biology

2.5.1. In vitro antileishmanial and antimalarial assays

Antileishmanial activity of the compounds was tested *in vitro* on a culture of *Leishmania donovani* promastigotes (strain S1). Compounds with appropriate dilution were added to a 96 well microplate with promastigotes (2×10^6 cell/mL) reaching final concentrations of 40, 8 and 1.6 μg/mL. The plates were incubated at 26 °C for 72 h and growth was determined by Alamar blue assay [38]. Pentamidine and Amphotericin B were used as standard antileishmanial agents.

All compounds were simultaneously tested for cytotoxicity against VERO (monkey kidney fibroblast) cells by Neutral Red assay [39], and IC_{50} values were computed from the growth inhibition curve.

Antimalarial activity was based on the effect on growth of asynchronous cultures of chloroquine sensitive (D6, Sierra Leone) and resistant (W2, IndoChina) strains of *Plasmodium falciparum*.

Appropriate dilutions of the compounds were prepared in DMSO or RPMI-1640 medium and added to cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) set up in clear, flat bottomed 96 well plates, with red blood cells and pure medium as controls. The plates were placed into the humidified chamber and flushed with a gas mixture of 90% N_2, 5% CO_2 & 5% O_2, and incubated at 37 °C for 48 h. Growth in each well was determined by parasite lactate dehydrogenase activity (pLDH) assay using Malstat® reagent [39]. Standard antimalarial agents chloroquine and artemisinin were used as the positive controls with DMSO as negative control.

3. Results and discussion

The key intermediate was synthesized from commercially available pregnelone, which was submitted to one-pot reductive amination with propargylamine, following standard procedure using $NaBH(OAc)_3$ and acetic acid in DCM (Scheme 1) [40].

Unfortunately, the reaction was very slow due to the low solubility of pregnenolone, affording the desired product as a mixture of isomers in only 25% yield after 10 days at room temperature. (Table 1, entry 1). It has been reported that ketosteroids can be converted in aminosteroids through reductive amination using $NaBH(OAc)_3$ in THF without AcOH [41]. Following that procedure and using 6 equivalents of propargyl amine, the yield was considerably improved, up to 75%. (Table 1, entry 2)

Different methodologies can be used to remove water from the reaction mixture, facilitating the formation of the key imine intermediate. We chose the addition of molecular sieves 4 Å, which improved the yield to 83%. (Table 1, entry 3). Finally, with addition of molecular sieves every 48 h, the reaction time was reduced to 6 days providing the expected propargylamino sterol **1** in almost quantitative yield (Table 1, entry 4). In all cases the reaction yielded a mixture of diasteroisomers which was resolved by column chromatography, with isomers **1*R*** and **1*S*** isolated in practically equal proportion, indicating no facial differentiation during imine reduction.

The isomer with higher R_f on TLC was crystallized from hexanes/ethyl acetate, and X-ray single-crystal diffraction studies showed *R* configuration for its C20 (Fig. 2).

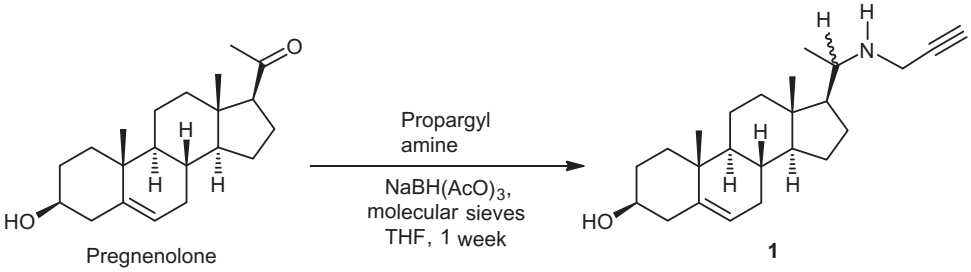
Having the key intermediate as a mixture of diastereomers could be a problem, ordinarily. In this case, however, it was advantageous, as the mixture was easily resolved and useful for our synthetic purposes. In fact, C20 is a key stereocenter for interaction with different enzymes involved in steroid metabolism in fungi and parasites [42]. These enzymes are, in consequence, druggable targets on which C20 diastereomers can be used for SAR studies.

Once the required key propargylamino intermediates were prepared, we started the diversity generation step preparing a pool of five different azides covering a variety of steric moieties including aryl, alkyl and isoprenyl substituents.

Azides were prepared by direct substitution of bromide with sodium azide on DMF except for geranyl azide, which was synthesized from geraniol using diphenylphosphoryl azide (DPPA) following Thompson's procedure [43]. Geranyl azide was found to actually be three chemical entities in equilibrium: tertiary azide, *E* and *Z* isomers of the primary azide (Scheme 2). The inseparable mixture was analyzed by ^{1}H NMR giving a ratio of 2-*E*: 2-*Z*: tertiary (5:3:1). The first studies on allyl azides were reported more than 50 years ago [44,45], proposing a [3,3]-sigmatropic rearrangement mediating the equilibrium among the species. That equilibrium is also temperature dependent, according to studies reported of their reactivity behavior and synthetic applications [46,47].

Despite the difficulties associated with working with such a complex mixture, we decided to move forward and use the isomeric mixture of geranyl azides, as they seemed to present an interesting approach to generate diversity.

Having a set of azides ready, we moved onto the preparation of a focused library of compounds through click chemistry. Reactions were conducted in a parallel solution synthesis setup under copper(II) sulphate catalytic conditions in water:*t*-BuOH (1:1) using sodium ascorbate as reductant [48]. The first set of five compounds was prepared using alkynyl sterol intermediate **1*R*** and azides (Scheme 3**)**. The reactions required excess of azide for completion and a reaction time of 18 h. All the products have 1,4-substitution on the 1,2,3,-triazol as expected, based on the original description



Scheme 1. Redutive amination of pregnenolone.

Table 1
Optimization of reaction conditions for reductive amination of pregnenolone.

Reagents	Eq.	Solvent	4 Å Molecular sieves	Time	Yield (%)
Propargylamine	1.2	DCM	–	10 days	**25**
$NaBH(AcO)_3$	2				
HAcO	1.8				
Propargylamine	6	THF	–	1 week	**72**
$NaBH(AcO)_3$	2				
Propargylamine	6	THF	One time	1 week	**83**
$NaBH(AcO)_3$	2				
Propargylamine	6	THF	Added every 2 days	6 days	**98**
$NaBH(AcO)_3$	2				

of this methodology and on the work of several other authors [25,49–53].

The reaction with the mixture of geranyl azides generated **2d-*R*** which was identified as an inseparable mixture of *E* and *Z* isomers, resulting solely from the reaction of the primary allylic azides, which is in accordance with the results obtained by Feldman et al. [46]. The *E*:*Z* ratio of **2d-*R*** was 60:40, determined by ^{1}H NMR and by comparison with previously prepared isoprenyl triazoles [54]. Final purified products, presented in Table 2, were obtained in good yields, with an 87% average for the **[2A-2E]-*R*** series.

Following the same procedure, **1*S*** was submitted to the same parallel click chemistry reactions with the azides. As before, the geranyl analog **2d-*S*** was a mixture with the same *E*:*Z* ratio. The average yield for the purified series **[2A-2E]-*S*** was 83%.

4. Biology

The final compounds were assayed against *P. falciparum*, D6, Sierra Leone (chloroquine sensitive), and W2, IndoChina (chloroquine resistant); against *Leishmania donovani*, the etiological agent of visceral leishmaniasis; and against the mammalian Vero cell line, for evaluation of cytotoxicity. Most of the compounds were poorly active against both strains of *P. falciparum*. The most active compound had IC_{50} around 4 μM, the average IC_{50} was around 6 μM, and the maximum concentration tested was 10 μM. Artemisinin and Chloroquine were used as controls, with IC_{50} below 90 nM. (Table 3)

Despite poor activity, there was a discernible pattern throughout the series that can be useful for future development of new libraries. In general, we can identify two populations of active compounds: **2C-*R***, **2D-*R*** and **2E-*R*** presented IC_{50} between 4.11 μM (**2D-*R*** – against D6) and 8.13 μM (**2E-*R*** against W2); **2C-*S***, **2D-*S*** and **2E-*S*** presented IC_{50} between 4.30 μM (**2D-*S*** – against W2) and 8.32 μM (**2E-*S*** against D6). Those compounds share long lipophilic substituents on the triazole; octyl, geranyl and phenyl propyl, and their activity seems to be independent of the C20 stereochemistry, but related to a triazole side chain longer than 5 carbons. A possible explanation for that behavior would be the compounds interacting non-specifically, without hitting a single molecular target, possibly involving sterol uptake pathways, which are vital for parasite survival and reproduction. However, non functionalized natural sterols, including phytosterols such as sitosterol and stigmasterol, did not show activity even at high concentrations [55], which prompted us to propose that

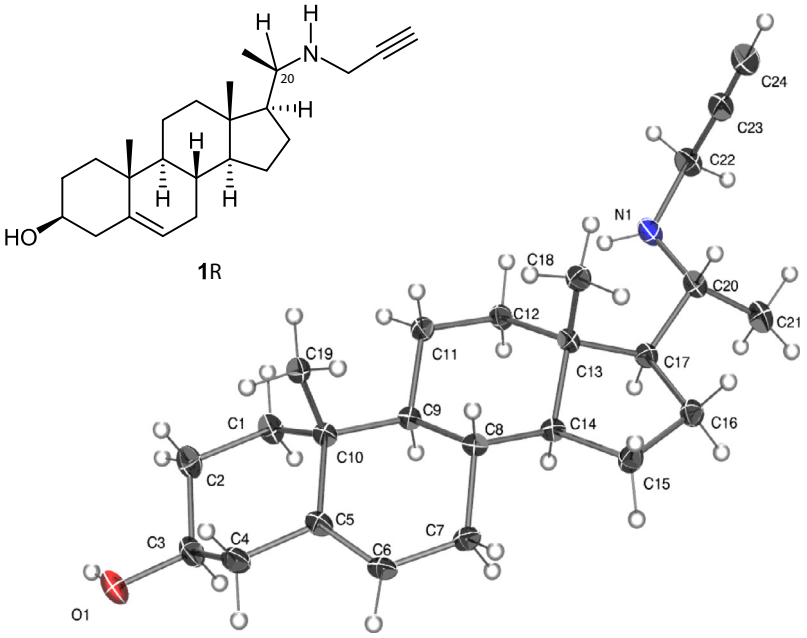
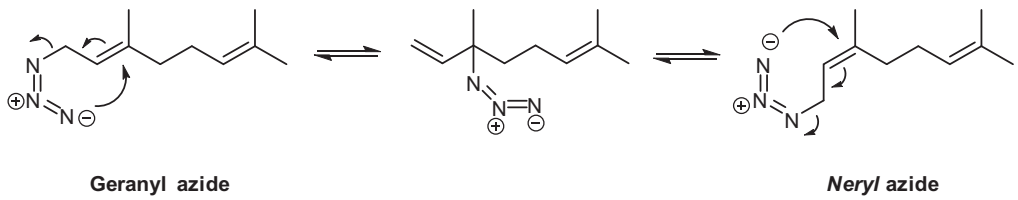
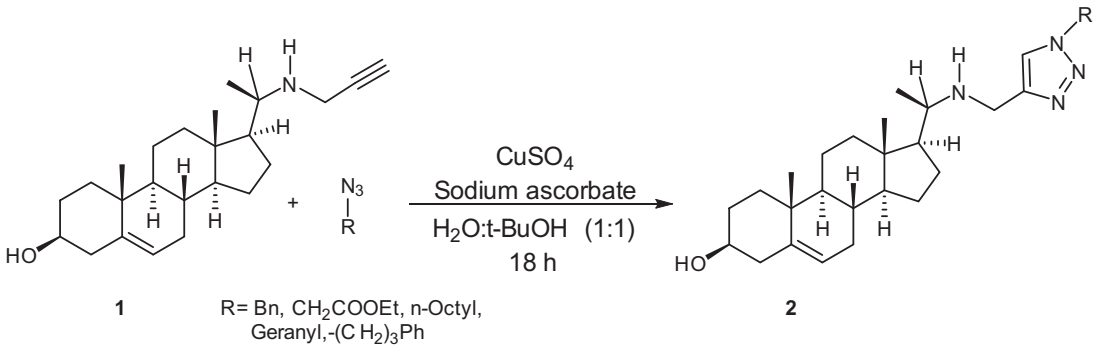


Fig. 2. Molecular structure of **1*R***.



Scheme 2. Geranyl azide rearrangement.



Scheme 3. Preparation of 1,2,3-triazoles by click chemistry.

Table 2
Yields of the final products prepared.

Compound	R	Yield (%)
2A-*R*	Bn	89
2B-*R*	CH_2COOEt	78
2C-*R*	Octyl	83
2D-*R*	Geranyl	90
2E-*R*	PhC_3H_6	94
2A-*S*	Bn	76
2B-*S*	CH_2COOEt	78
2C-*S*	Octyl	75
2D-*S*	Geranyl	94
2E-*S*	PhC_3H_6	90

87% average yield R series.
83% average yield S series.

Table 3
Antiparasitic activity of products and intermediates.

Compound	*L. donovani* IC_{50} μM	*P. falciparum* (D6 strain) IC_{50} μM	*P. falciparum* (W2 strain) IC_{50} μM
1*R*	>20	>10.0	>10.0
2A-*R*	6.14	>10.0	>10.0
2B-*R*	6.60	>10.0	>10.0
2C-*R*	2.55	4.70	5.87
2D-*R*	1.14	4.11	4.49
2E-*R*	1.94	6.19	8.13
1-*S*	4.50	>10.0	>10.0
2A-*S*	1.33	9.62	8.59
2B-*S*	6.40	>10.0	>10.0
2C-*S*	1.47	6.26	4.50
2D-*S*	1.68	8.79	4.30
2E-*S*	1.35	8.32	5.42
Pentamidine	6.17		
Amphotericin B	0.35		
Chloroquine		0.083	0.422
Artemisinin		0.094	0.094

triazolylsterols have a specific molecular target or a combination of mechanisms of action responsible for improved activity in malaria, compared to simple sterols with long side chains. In fact, it has also been reported that antiplasmodial sterols isolated from *S. nudum*, functionalized with long ketonic/hydroxylic side chains, produce oxidative stress and inhibited the heme polymerization process [56]. Also, it is important to remark that propargyl intermediates **1*R*** and **1*S*** were inactive against *P. falciparum*, suggesting than the combination of the amino group and a triazol with a long non-polar substituent constitutes a pharmacophoric group for a target yet to be identified.

All compounds (except **1-*R***) showed good activity against *L. donovani* promatigotes (Table 3). The less active compound, **2B-*R***, showed IC_{50} of 6.60 μM, and the most active, **2D-*R*** showed IC_{50} of 1.14 μM. **2D-*R*** is more than 5 times more active than the control drug pentamidine, and a little over 3 times less active than Amphotericin B. The same pattern of activity observed against *P. falciparum* was observed against *L. donovani*, in which the presence of a long chain substituent on the triazol increased activity. **2C**, **2D**, and **2E**, both ***R*** and ***S*** had their IC_{50} values distributed within a narrow range of 1.4 μM. The most active compound, **2D-*R***, is a mixture of two regioisomers and will require further efforts to prepare and assay both isomers independently. The propargyl intermediate **1*R*** was also inactive against *L. donovani*, but **1*S*** was active with an IC_{50} of 4.50 μM, suggesting a possible influence of the configuration of the C20 stereocenter, along with an independent activity of the amino group, further enhanced by the incorporation of the triazole moiety. None of the compounds reported showed cytotoxicity towards mammalian kidney fibroblasts (vero) cells at concentration upto 4.75 μg/mL.

Antileishmanial activity is comparable with other trypanocidal sterols, such as azasterols, which have been synthesized as inhibitors of sterol methyltransferase, an enzyme that has been validated as a target for leishmanicidal and trypanocidal drugs. It has been postulated that azasterols can mimic the carbocation mechanistic intermediate and Gilbert's group has explored that hypothesis preparing compounds containing nitrogen on the sterol lateral chain [17,19]. These compounds showed an enzyme inhibition profile well correlated with their antiparasitic activity, validating sterol methyltransferase as a druggable target. Based on the structural similarities between Gilbert's compounds and ours, it is possible that 1,2,3-triazolyl sterols can also interfere with the activity of sterol methyltransferase, and we plan on testing this hypothesis as we continue our work with triazolylsterols. Our future plans include testing these compounds against the ethiological agents of Chagaś disease (*T. cruzi)* and human African trypanosomiasis (*Trypanosoma brucei*).

5. Conclusions

We have prepared a small library of triazolylsterols by reductive amination of commercially available pregnenolone, followed by Cu(I) catalyzed cycloaddition to form amino triazolyl derivatives. The compounds showed marginal activity against two strains of *P. falciparum*, but very promising activity against *L. donovani*, with the most active compound being 5 times more active than one of the control drugs. These results are encouraging, as many drugs were developed from initial lead compounds active at the micromolar level, and subsequently modified to reach improved activity and biopharmaceutical properties, moving then forward

in the drug development process. Our triazolyl sterols have the advantage of a simple preparation, and the possibility that their action involves a mechanism different from currently known compounds, which encourages further mechanistic studies and additional exploration of structural modifications, such as different chain length and composition of the spacer between the amino group and the triazole moiety, or different choices of substituent on the triazole. We also intend to study the effect of triazolylsterols on other stages of kinetoplastid protozooa life cycles, and to explore synergism with current therapeutic drugs.

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Appendix A. Supplementary data

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References

[1] Austin DH. Research and development in the pharmaceutical industry. Congress of the United States – Congressional Budget, Office; 2006. p. 55.
[2] WHO. Making a difference – 30 years of research and capacity building in tropical diseases; 2007.
[3] Feasey N, Wansbrough-Jones M, Mabey DCW, Solomon AW. Neglected tropical diseases. Br Med Bull 2010;93:179–200.
[4] Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, et al. A research agenda to underpin malaria eradication. PLoS Med 2011;8:e1000406.
[5] Research priorities for chagas disease, human African trypanosomiasis and leishmaniasis. WHO Tech Rep Ser. Geneva; 2012.
[6] Cohen J, Dibner MS, Wilson A. Development of and access to products for neglected diseases. PLoS One 2010;5:e10610.
[7] Hanson JR. Steroids: partial synthesis in medicinal chemistry. Nat Prod Rep 2010;27:887–99.
[8] Heasley B. Chemical synthesis of the cardiotonic steroid glycosides and related natural products. Chem Eur J 2012;18:3092–120.
[9] Kovganko NV, Ananich SK. The chemical synthesis of sterols: latest advances. Chem Nat Compd 1999;35:229–59.
[10] Abdelhalim MM, El-Saidi MMT, Rabie ST, Elmegeed GA. Synthesis of novel steroidal heterocyclic derivatives as antibacterial agents. Steroids 2007;72:459–65.
[11] Thomas ST, Yang X, Sampson NS. Inhibition of the M. tuberculosis 3β-hydroxysteroid dehydrogenase by azasteroids. Bioorg Med Chem Lett 2011;21:2216–9.
[12] Dantas-Leite L, Urbina JA, de Souza W, Vommaro RC. Selective anti-Toxoplasma gondii activities of azasterols. Int J Antimicrob Agents 2004;23:620–6.
[13] Croft SL, Coombs GH. Leishmaniasis – current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol 2003;19:502–8.
[14] Roberts CW, McLeod R, Rice DW, Ginger M, Chance ML, Goad LJ. Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. Mol Biochem Parasitol 2003;126:129–42.
[15] Pan L, Lezama-Davila CM, Isaac-Marquez AP, Calomeni EP, Fuchs JR, Satoskar AR, et al. Sterols with antileishmanial activity isolated from the roots of Pentalinon andrieuxii. Phytochemistry 2012;82:128–35.
[16] Kittakoop P, Suttisri R, Chaichantipyuth C, Vethchagarun S, Suwanborirux K. Norpregnane glycosides from a thai soft coral, Scleronephthya pallida. J Nat Prod 1998;62:318–20.
[17] Gros L, Lorente SO, Jimenez CJ, Yardley V, Rattray L, Wharton H, et al. Evaluation of azasterols as anti-parasitics. J Med Chem 2006;49:6094–103.
[18] Song Z, Nes WD. Sterol biosynthesis inhibitors: potential for transition state analogs and mechanism-based inactivators targeted at sterol methyltransferase. Lipids 2007;42:15–33.
[19] Magaraci F, Jimenez-Jimenez C, Rodrigues C, Rodrigues JCF, Braga MV, Yardley V, et al. Azasterols as inhibitors of sterol 24-methyltransferase in leishmania species and *Trypanosoma cruzi*. J Med Chem 2003;46:4714–27.
[20] Spandl RJ, Bender A, Spring DR. Diversity-oriented synthesis; a spectrum of approaches and results. Org Biomol Chem 2008;6:1149–58.
[21] Harris CJ, Hill RD, Sheppard DW, Slater MJ, Stouten PF. The design and application of target-focused compound libraries. Comb Chem High Throughput Screen 2011;14:521–31.
[22] Agalave SG, Maujan SR, Pore VS. Click chemistry: 1,2,3-triazoles as pharmacophores. Chem – Asian J 2011;6:2696–718.
[23] Tron GC, Pirali T, Billington RA, Canonico PL, Sorba G, Genazzani AA. Click chemistry reactions in medicinal chemistry: applications of the 1,3-dipolar cycloaddition between azides and alkynes. Med Res Rev 2008;28:278–308.
[24] Moses JE, Moorhouse AD. The growing applications of click chemistry. Chem Soc Rev 2007;36:1249–62.
[25] Kumar D, Reddy VB, Kumar A, Mandal D, Tiwari R, Parang K. Click chemistry inspired one-pot synthesis of 1,4-disubstituted 1,2,3-triazoles and their Src kinase inhibitory activity. Bioorg Med Chem Lett 2011;21:449–52.
[26] Maurya SK, Gollapalli DR, Kirubakaran S, Zhang M, Johnson CR, Benjamin NN, et al. Triazole inhibitors of *Cryptosporidium parvum* inosine 5′-monophosphate dehydrogenase. J Med Chem 2009;52:4623–30.
[27] Zhang X, He Y, Liu S, Yu Z, Jiang Z-X, Yang Z, et al. Salicylic acid based small molecule inhibitor for the oncogenic Src homology-2 domain containing protein tyrosine phosphatase-2 (SHP2). J Med Chem 2010;53:2482–93.
[28] Campo VL, Sesti-Costa R, Carneiro ZA, Silva JS, Schenkman S, Carvalho I. Design, synthesis and the effect of 1,2,3-triazole sialylmimetic neoglycoconjugates on *Trypanosoma cruzi* and its cell surface trans-sialidase. Bioorg Med Chem 2012;20:145–56.
[29] Sharling L, Liu X, Gollapalli DR, Maurya SK, Hedstrom L, Striepen B. A screening pipeline for antiparasitic agents targeting cryptosporidium inosine monophosphate dehydrogenase. PLoS Negl Trop Dis 2010;4:e794.
[30] Capes A, Patterson S, Wyllie S, Hallyburton I, Collie IT, McCarroll AJ, et al. Quinol derivatives as potential trypanocidal agents. Bioorg Med Chem 2012;20:1607–15.
[31] Labadie GR, de la Iglesia A, Morbidoni HR. Targeting tuberculosis through a small focused library of 1,2,3-triazoles. Mol Divers 2011;15:1017–24.
[32] Gill C, Jadhav G, Shaikh M, Kale R, Ghawalkar A, Nagargoje D, et al. Clubbed [1,2,3] triazoles by fluorine benzimidazole: a novel approach to H37Rv inhibitors as a potential treatment for tuberculosis. Bioorg Med Chem Lett 2008;18:6244–7.
[33] Stefely JA, Palchaudhuri R, Miller PA, Peterson RJ, Moraski GC, Hergenrother PJ, et al. N-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)arylamide as a new scaffold that provides rapid access to antimicrotubule agents: synthesis and evaluation of antiproliferative activity against select cancer cell lines. J Med Chem 2010;53:3389–95.
[34] Banday AH, Shameem SA, Gupta BD, Kumar HM. D-ring substituted 1,2,3-triazolyl 20-keto pregnenanes as potential anticancer agents: synthesis and biological evaluation. Steroids 2010;75:801–4.
[35] Deobald AM, Camargo LRS, Alves D, Zukerman-Schpector J, Corrêa AG, Paixão MW. Click chemistry: an efficient synthesis of heterocycles substituted with steroids, saponins, and digitalis analogues. Synthesis 2011:4003–10.
[36] Frank E, Molnar J, Zupko I, Kadar Z, Wolfling J. Synthesis of novel steroidal 17alpha-triazolyl derivatives via Cu(I)-catalyzed azide-alkyne cycloaddition, and an evaluation of their cytotoxic activity in vitro. Steroids 2011;76:1141–8.
[37] TLC staining solution: TLC plate was dipped into a solution of 2 mL anisaldehyde, 10 mL glacial acetic acid and 5 mL sulfuric acid in 340 mL ethanol followed by heating with a heat gun.
[38] Mikus J, Steverding D. A simple colorimetric method to screen drug cytotoxicity against Leishmania using the dye Alamar Blue®. Parasitol Int 2000;48:265–9.
[39] Babich H, Borenfreund E. Cytotoxicity of T-2 toxin and its metabolites determined with the neutral red cell viability assay. Appl Environ Microbiol 1991;57:2101–3.
[40] Abdel-Magid AF, Carson KG, Harris BD, Maryanoff CA, Shah RD. Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride. Studies on direct and indirect reductive amination procedures1. J Org Chem 1996;61:3849–62.
[41] Nawaz Khan S, Bae S-Y, Kim H-S. A highly stereoselective reductive amination of 3-ketosteroid with amines: an improved synthesis of 3α-aminosteroid. Tetrahedron Lett 2005;46:7675–8.
[42] Kanagasabai R, Zhou W, Liu J, Nguyen TT, Veeramachaneni P, Nes WD. Disruption of ergosterol biosynthesis, growth, and the morphological transition in *Candida albicans* by sterol methyltransferase inhibitors containing sulfur at C-25 in the sterol side chain. Lipids 2004;39:737–46.
[43] Thompson AS, Humphrey GR, DeMarco AM, Mathre DJ, Grabowski EJJ. Direct conversion of activated alcohols to azides using diphenyl phosphorazidate. A practical alternative to Mitsunobu conditions. J Org Chem 1993;58:5886–8.
[44] VanderWerf CA, Heasley VL. Preparation and rearrangements. J Org Chem 1966;31:3534.
[45] Gagneuz A, Winstein S, Young WG. Rearrangement of allyl azides. J Org Chem 1969;1:5956–7.
[46] Feldman AK, Colasson B, Sharpless KB, Fokin VV. The allylic azide rearrangement: achieving selectivity. J Am Chem Soc 2005;127:13444–5.
[47] Fava C, Galeazzi R, Mobbili G, Orena M. Steric constraints against [3,3]-sigmatropic rearrangement of allylic azides. A convenient approach to

b-L-4-aminopent-2-enoglyceropyranosides. Tetrahedron: Assymetry 2001;12:2731–41.
[48] Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew Chem Int Ed Engl 2002;41:2596–9.
[49] Vasilevsky SF, Govdi AI, Sorokina IV, Tolstikova TG, Baev DS, Tolstikov GA, et al. Rapid access to new bioconjugates of betulonic acid via click chemistry. Bioorg Med Chem Lett 2011;21:62–5.
[50] Trabocchi A, Menchi G, Cini N, Bianchini F, Raspanti S, Bottoncetti A, et al. Click-chemistry-derived triazole ligands of arginine–glycine–aspartate (RGD) integrins with a broad capacity to inhibit adhesion of melanoma cells and both in vitro and in vivo angiogenesis. J Med Chem 2010;53:7119–28.
[51] Kamal A, Shankaraiah N, Devaiah V, Laxma Reddy K, Juvekar A, Sen S, et al. Synthesis of 1,2,3-triazole-linked pyrrolobenzodiazepine conjugates employing 'click' chemistry: DNA-binding affinity and anticancer activity. Bioorg Med Chem Lett 2008;18:1468–73.
[52] Hausner SH, Marik J, Gagnon MKJ, Sutcliffe JL. In vivo positron emission tomography (PET) imaging with an αvβ6 specific peptide radiolabeled using ^{18}F-"click" chemistry: evaluation and comparison with the corresponding 4-[^{18}F]fluorobenzoyl- and 2-[^{18}F]fluoropropionyl-peptides. J Med Chem 2008; 51:5901–4.
[53] Godeau G, Staedel C, Barthélémy P. Lipid-conjugated oligonucleotides via "click chemistry" efficiently inhibit hepatitis C virus translation. J Med Chem 2008;51:4374–6.
[54] Porta EOJ, Bracca ABJ, Labadie GR. Unpublished results; 2013.
[55] Ochieng CO, Manguro LA, Owuor PO, Akala H. Voulkensin C-E, new 11-oxocassane-type diterpenoids and a steroid glycoside from Caesalpinia volkensii stem bark and their antiplasmodial activities. Bioorg Med Chem Lett 2013;23:3088–95.
[56] Pabon A, Deharo E, Blair S. *Plasmodium falciparum*: solanum nudum SN-1 steroid antiplasmodial activity when combined with antimalarial drugs. Exp Parasitol 2011;127:222–7.