



## Original article

## Derivatives of grindelic acid: From a non-active natural diterpene to synthetic antitumor derivatives



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## ABSTRACT

Using several reactions that include homologations and asymmetric epoxidations as well as Ugi and Huisgen couplings, we generated a small focused library of new derivatives from the labdane-type diterpene grindelic acid. These compounds were evaluated as cytotoxic agents against a panel of five human solid tumor cell lines (HBL-100, HeLa, SW1573, T-47D, and WiDr). The presence of the diamide functionalizations enhanced the cytotoxic effect. *N*-Benzyl-*N*-(1-(benzylamino)-2-methyl-1-oxopropan-2-yl)grindelicamide, proved to be the most active product in all cell lines tested, with values of 0.95 ( $\pm 0.38$ )  $\mu$ M against HBL-100 cells.

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## 1. Introduction

The plant kingdom comprise a relatively small group within biodiversity, with about 250,000 species, of which near 6%, has been studied for their biological activity, and only about 15% has been studied in a phytochemical way. Arguably, the plant kingdom is the largest source of structural diversity in organic compounds, forming a reservoir of chemical structures which is not possible to find in another environment. In this sense, a large number of natural products isolated from plants (or their transformed products) are known, to have relevant biological activities showing an important applicative potential [1].

The quest for new chemical structures with antiproliferative properties, which can be used in the future as chemotherapeutic agents, is an active area of research stimulated by the discovery of new biological targets and the possibility of arising new drugs without undesirable side effects. In the past years, there has been an

increasing interest in the development of new pharmaceuticals based on a nucleus present in natural products along with the increase of life-threatening diseases such as AIDS, cancer, hepatitis, etc. [2]. It is noteworthy that some semi synthetic compounds derived from a natural framework sometimes show higher bioactivity than the original natural product. Taking into account their broad range of biological activities, such as enzyme inducing, modulation of immune cell function, and cytotoxic against human tumor cell lines [3–6], labdane diterpenes have attracted scientific interest.

Analyzing the structure of molecules used in cancer therapy, most of them show nitrogenated functional groups in their framework like amide and carbamate moieties [7]. Recently, it has been reported a series of disubstituted 1,2,3-triazole exhibiting potent cytotoxicity in the nanomolar range and tubulin inhibitory activity in the low micromolar range [8]. This kind of compounds is considered an interesting unit in the design of anticancer drugs. Such heterocycle may act due to their dipolar character, rigidity, ability to form hydrogen bridge bonds or as simple connectors [9].

Synthetic organic chemists have gained great interest for 1,2,3-triazoles in the development of new biologically active molecules [10]. The triazole moiety does not occur in nature, but 1,2,3-triazole

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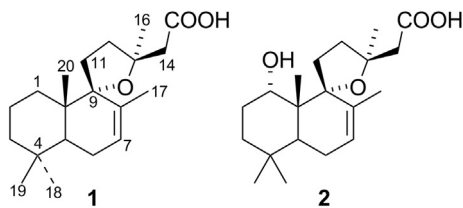


Fig. 1. Grindelic acid and 1-( $\alpha$ )-hydroxygrindelic acid.

cores may form the basis of small-molecule pharmaceutical leads. Molecules containing this heterocyclic nucleus have been reported having anti-HIV, antimicrobial, anti-allergic, antifungal and anti-tumor activities [10].

Grindelic acid (**1**) is a labdane-type diterpene that was reported as the main secondary metabolite from *Grindelia chilensis* Cabr and *Grindelia pulchella* Dunal var. *pulchella* (Asteraceae). This compound possesses a suitable C-14-side-chain for inserting functionalities carrying nitrogen and oxygen [11]. In a previous work, we have reported about the enhancement of the cytotoxicity of some sesquiterpenes and iridoids when their lipophilicity was increased by the introduction of alkyl and/or aryl-silyl functionalities on the natural framework [12].

In consideration of the above mentioned factors, herein we describe our findings aimed to the synthesis and cytotoxic evaluation of oxygenated and oxy-nitrogenated derivatives from grindelic acid (**1**). It should be emphasized that this diterpene can be isolated in significant quantities from the aforementioned natural sources. Furthermore, these plants have a wide distribution in the Patagonian steppe zones of Argentina and Chile [13].

## 2. Result and discussion

### 2.1. Chemistry

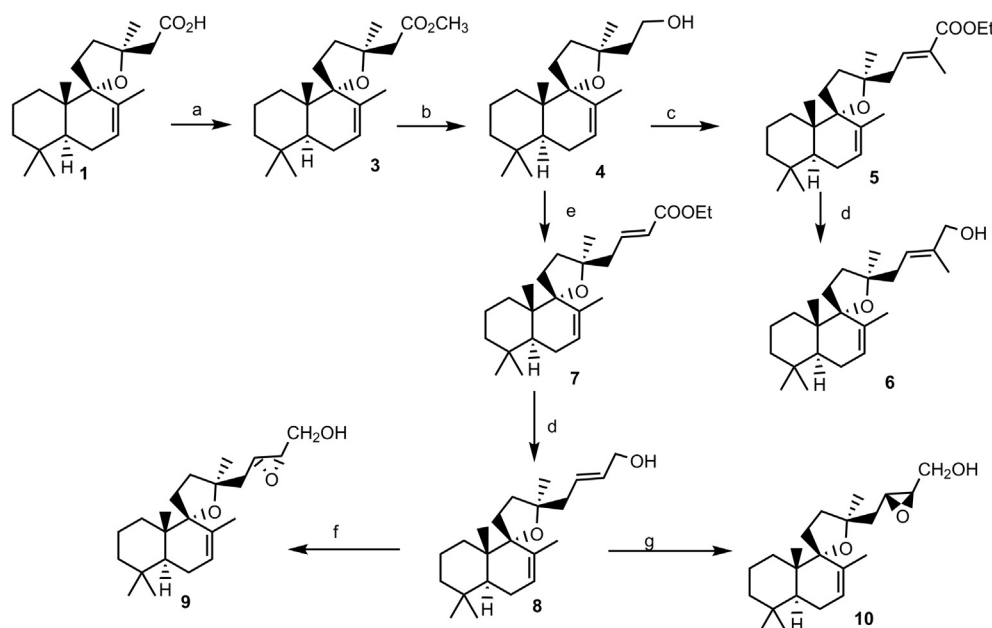
From *G. pulchella* Dunal var. *pulchella*, we obtained the metabolites grindelic acid (**1**) and 1 $\alpha$ -hydroxygrindelic acid (**2**), as reported earlier (Fig. 1) [13].

In preliminary tests, compound **1** (main secondary metabolite) exhibited greater bioactivity than **2**. Therefore, it was chosen as a starting material for the preparation of a series of thirty-six derivatives. Firstly, we prepared several oxygenated compounds **3–10** taking advantage of the presence of the carboxylic acid group (Scheme 1). Methyl ester derivative **3** was obtained from **1** by direct esterification under standard conditions. Subsequent reduction of the ester group at the side chain, with  $\text{LiAlH}_4$  gave alcohol **4** in high yields. The oxidation of **4** under Parikh–Doering conditions and subsequent Wittig reaction using ethyl 2-(triphenylphosphoranylidene)-propanoate, led to the  $\alpha,\beta$ -unsaturated ester **5** that after reduction with DIBAL-H afforded in good yields alcohol **6**. Following the same sequence with ethyl(triphenylphosphoranylidene)acetate, compounds **7** and **8**, were obtained from **4**. Epoxides **9** and **10** were achieved by Katsuki–Sharpless asymmetric epoxidation of the allylic alcohol **8** using *R,R*-(+)-DET and *S,S*-(-)-DET, respectively.

Cycloaddition and multicomponent reactions are convergent procedures of high synthetic utility combining chemical and atomic efficiency. Within the first type, azide–alkyne Huisgen cycloaddition is a powerful tool to prepare 1,2,3-triazoles in a straightforward manner [14]. Multicomponent Reactions (MCRs) are convergent reactions in which three or more starting materials react to form a product where, basically, all or most of the atoms contribute to the newly formed product. In MCRs a product is assembled according to a cascade of elementary chemical reactions. Thus, there is a network of reaction equilibrium which all finally flow into an irreversible step yielding the product. In this work, it was prepared a series of oxy-nitrogenated derivatives, compounds **11–22** by Ugi reaction, **27–32** and **37** by Huisgen cycloadditions, and **23–26** and **33–36** were prepared by classical chemistry. Noteworthy, in all proposals the natural product stereochemistry remained intact.

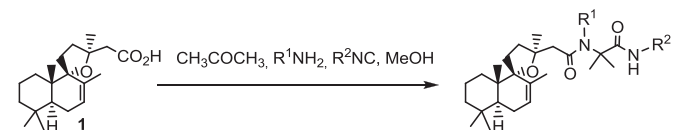
Derivatives **11–22** were obtained by the Ugi reaction using **1** as carboxylic acid and acetone as a source of carbonyl group. Benzylamine (compounds **11–17**), and aniline (compounds **18–22**) were used as amines varying the corresponding isocyanides (Table 1).

One of the most popular reactions within the click chemistry paradigm is the Cu(I)-catalyzed 1,3-dipolar Huisgen cycloaddition of alkynes and azides. This reaction proceeds with great efficiency



Scheme 1. Reagents and conditions: a)  $\text{CH}_2\text{N}_2$ ,  $\text{Et}_2\text{O}$ , rt, 94%; b)  $\text{LiAlH}_4$ , THF, 70 °C, 91%; c)  $\text{SO}_3 \cdot \text{Py}$ ,  $\text{Et}_3\text{N}$ , DMSO,  $\text{Ph}_3\text{P}=\text{C}(\text{CH}_3)\text{CO}_2\text{Et}$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 74%; d) DIBAL-H,  $\text{Et}_2\text{O}$ , 0 °C, 82–89%; e)  $\text{SO}_3 \cdot \text{Py}$ ,  $\text{Et}_3\text{N}$ , DMSO,  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Et}$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 94%; f)  $\text{Ti}(\text{OPr-}i)_4$ , *R,R*-(+)-DET, TBHP,  $\text{CH}_2\text{Cl}_2$ , –20 °C, 71%; g)  $\text{Ti}(\text{OPr-}i)_4$ , *S,S*-(-)-DET, TBHP,  $\text{CH}_2\text{Cl}_2$ , –20 °C, 85%.

**Table 1**  
Diamides obtained from the multicomponent Ugi reaction of grindelic acid (**1**).<sup>a</sup>



Compound	R <sup>1</sup>	R <sup>2</sup>	Yield <sup>b</sup>
11			59
12			75
13			71
14			63
15			79
16			79
17			97
18			78
19			26
20			23
21			43
22			92

<sup>a</sup> All the reactions were carried out using 1 equiv of compound **1**, 1 equiv of the corresponding amine, 1.2 equiv of propanone and 1.1 equiv of the corresponding isocyanide.

<sup>b</sup> Isolated yield.

and selectivity in aqueous media and yields a triazole moiety [15]. Our first objective was to obtain azide **23** and alkyne **26**, using as starting material alcohol **4** (see Scheme 2).

The coupling of azide **23** and various commercial alkynes, under Huisgen conditions, provided the 1,2,3-triazoles **27–28** in good yields. In a similar manner, the quasi symmetrical hybrid **29** was prepared from azide **23** and alkyne **26** (Table 2).

Using Huisgen conditions and adjusting 1 equivalent of the suitable di-alkyne per 2 equivalents of the azide **23**, the dimers **30–32** were achieved (see Table 3). Alkynes used for the preparations of compounds **30** and **31** are commercially available, and the alkyne used to arrive to compound **32** was synthesized according to the procedure described in the literature [16].

The final set of derivatives was prepared with the aim to introduce the maximum of structural diversity at the final hybrid compounds. Thus, carbonyl imidazole **33** was prepared by the reaction of alcohol **4** with carbonyl diimidazole (CDI) in CH<sub>2</sub>Cl<sub>2</sub>. Derivatives **34–35** were obtained from compound **33** by treatment

with diverse amines. The use of ethylenediamine provided dimeric carbamate **36**. Finally, Huisgen coupling of **35** with azide **23** yielded the hybrid triazole **37** (Scheme 3).

## 2.2. Antiproliferative activity

The *in vitro* antiproliferative activity was evaluated using the National Cancer Institute (NCI) protocol after 48 h of drug exposure using the sulforhodamine B (SRB) assay. The results, expressed as GI<sub>50</sub> values, are shown in Table 4. Derivatives **20–22**, **31**, and **36** were not evaluated due to their low solubility in DMSO under the testing conditions.

From the growth inhibition results we can depict some preliminary structure–activity relationships. The group of oxygenated derivatives (**3–10**) is more active than the natural products (**1–2**) where they derive from, having GI<sub>50</sub> values in all cell lines in the range 16–37 μM. In this series, compounds **5** and **7**, both bearing an ethyl ester in the side chain, gave higher GI<sub>50</sub> values (>30 μM) in the most resistant cell lines T-47D and WiDr. When compared to the methyl ester analog **3**, one could speculate with the bulkiness of the ester group on the side chain and its reverse effect on the activity. However, when the side chain was elongated through an Ugi reaction (compounds **11–22**) a clear enhancement in the antiproliferative activity was observed. In fact, compound **17** resulted the most active compound of the whole study with GI<sub>50</sub> values in the range 0.95–1.9 μM. In this second group of compounds, a relationship between substituents and biological activity could not be established. When considering the derivatives of the Huisgen reaction **27–29**, no improvement was observed in the activity when compared to the most active derivative **17**. Finally, for the analogs bearing two grindelic scaffolds (**29–32** and **36–37**), the activity profile was variable. Thus, derivatives **32** and **37** were inactive (GI<sub>50</sub> > 100 μM), whilst **30** and **34** showed GI<sub>50</sub> values <10 μM against all cell lines tested.

## 3. Conclusion

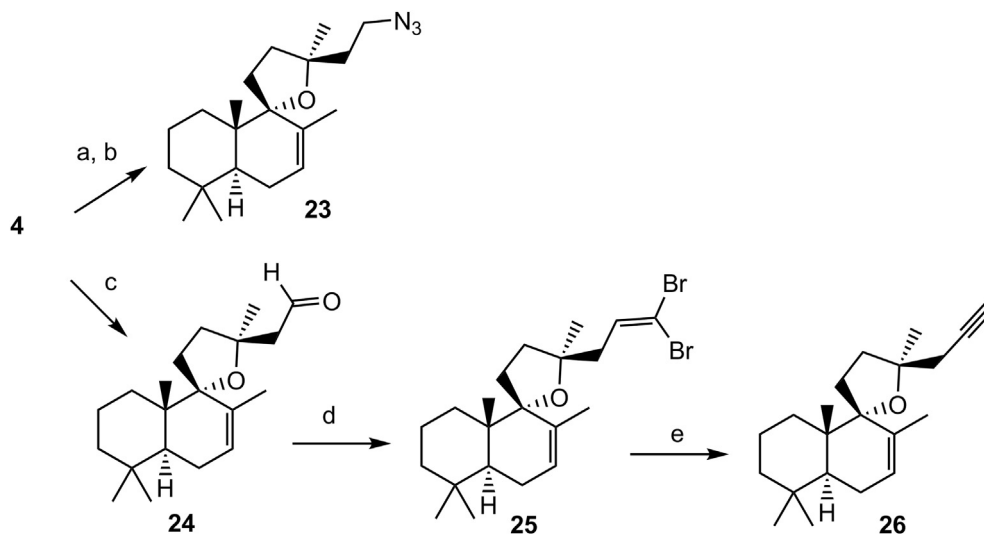
Given these results, the use of a natural product can be a viable strategy to prepare new active molecules. With adequate quantities of a natural product, and using accessible synthetic routes, it has been possible to obtain new structures exhibiting interesting bioactivities as antitumor agents. Grindelic acid (**1**) was the starting material for the transformation into several oxygenated, and oxy-nitrogenated moieties at the C-14 side chain by means of diverse reactions. Our strategy allowed to reach thirty-five new compounds including functionalities such as epoxides, diamides, carbamates and 1,2,3-triazoles, among others. A significant number of these synthetic derivatives showed higher activity than the natural product. It is noteworthy that the oxy-nitrogenated and nitrogenated systems were more active than oxygenated ones, being the diamides the most active compounds. The *N*-benzyl-*N*-(1-(benzylamino)-2-methyl-1-oxopropan-2-yl) amide of grindelic acid (**17**) proved to be the most active product in all cell lines tested.

Finally, this work aims to demonstrate the possibility of achieving new antitumor leaders from molecular frameworks provided by nature, getting sometimes a dramatic enhance of the bioactivity through usual chemical transformations.

## 4. Experimental

### 4.1. Chemistry

Unless stated otherwise, all solvents were purified by standard techniques. Reactions requiring anhydrous conditions were performed under argon. Anhydrous magnesium sulfate was used for



**Scheme 2.** Reagents and conditions: a) MsCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 12 h, 90%; b) NaN<sub>3</sub>, DMF, 40 °C, 48 h, 80%; c) (COCl)<sub>2</sub>, Et<sub>3</sub>N, DMSO, Cl<sub>2</sub>CH<sub>2</sub>, -78 °C, 30 min, 99%; d) PPh<sub>3</sub>, CBr<sub>4</sub>, Et<sub>3</sub>N, Cl<sub>2</sub>CH<sub>2</sub>, 0 °C, 15 min; e) *n*-BuLi, THF, -78 °C, 30 min, 53%.

drying solutions. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), and were visualized by use of UV light, 2.5% phosphomolybdic acid in ethanol or vanillin with acetic and sulfuric acid in ethanol with heating [17]. Purification was performed by column chromatography on silica gel (230–400 mesh) using *n*-hexane and ethyl acetate gradient as solvent. <sup>1</sup>H NMR spectra were recorded on a Bruker 500 or 400 MHz, <sup>13</sup>C NMR spectra were recorded at 100 MHz, and chemical shifts are reported relative to internal Me<sub>4</sub>Si ( $\delta = 0$ ). Melting points were determined by using an Electrothermal IA9000 melting point apparatus, are reported in degrees Celsius and are uncorrected. Optical rotations were recorded in a Perkin Elmer polarimeter 343. High resolution ESI mass spectra were obtained from a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, an RF-only

hexapole ion guide, and an external electrospray ion source. HRMS spectra were obtained on a mass spectrometer Micromass AutoSpec.

#### 4.1.1. Preparation of grindelic acid methyl ester (**3**)

Grindelic acid (**1**) (10 g, 31.2 mmol) was dissolved in 124 mL of dry Et<sub>2</sub>O (0.25 M) and was cooled at 0 °C. A solution of CH<sub>2</sub>N<sub>2</sub> in Et<sub>2</sub>O (1 M) was added dropwise until the formation of N<sub>2</sub> disappear. Reaction was checked by TLC. A few drops of acetic acid were added to quench the excess of diazomethane. Solvent was evaporated and the crude material was purified by silica gel chromatography to obtain 9.85 g, as yellow oil of **3**, (29.46 mmol, 94% yield). [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -117.2 (c 8.1, MeOH); IR  $\nu_{\text{max}}$ , cm<sup>-1</sup> (KBr): 3030, 2922, 1749 (C=O), 1672 (C=C), 991 (=C-H); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  5.52 (s, 1H, H-7), 3.67 (s, 3H, OCH<sub>3</sub>), 2.75 (d, *J* = 14, 1H, H-14 proR), 2.64 (d, *J* = 14, 1H, H-14 proS), 2.22 (m, 1H, H-12A), 2.07 (m, 2H, H-6A and H-11A), 1.87 (m, 3H, H-1 and H-12B), 1.84 (m, 2H, H-6B and H-11B), 1.79 (s, 3H, H-17), 1.66 (m, 3H, H-3 and H-5), 1.39 (m, 2H, H-2), 1.36 (s, 3H, H-16), 0.92 (s, 3H, H-19), 0.89 (s, 3H, H-18), 0.84 (s, 3H,

**Table 2**  
1,2,3-Triazole obtained by Huisgen reaction using mono-alkynes.<sup>a</sup>

Compound	R	Yield <sup>b</sup>
<b>27</b>		48
<b>28</b>		78
<b>29</b>		64

<sup>a</sup> All the reactions were carried out using the azide **23** (1 equiv), alkyne (1 equiv), sodium ascorbate (0.3 equiv) and copper(II) sulfate pentahydrate (0.12 equiv) in a 1:1 mixture of water and ethanol (0.1 M).

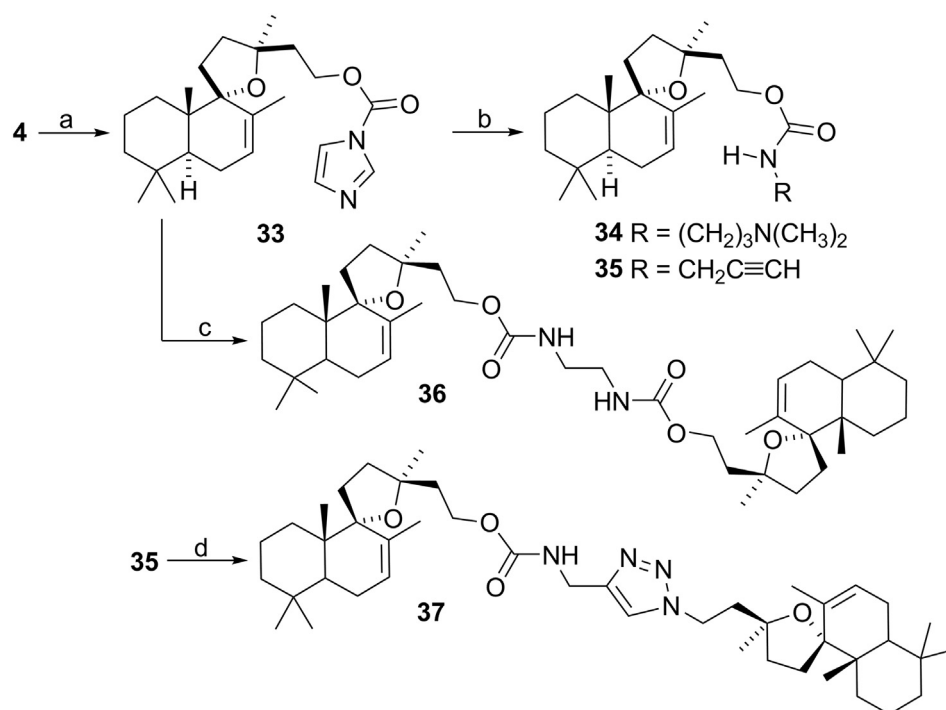
<sup>b</sup> Isolated yield.

**Table 3**  
1,2,3-Triazole obtained by Huisgen reaction using di-alkynes.<sup>a</sup>

Compound	R	Yield <sup>b</sup>
<b>30</b>		64
<b>31</b>		51
<b>32</b>		46

<sup>a</sup> All the reactions were carried out using the azide **23** (1 equiv), di-alkyne (0.5 equiv), sodium ascorbate (0.3 equiv) and copper(II) sulfate pentahydrate (0.12 equiv) in a 1:1 mixture of water and ethanol (0.1 M).

<sup>b</sup> Isolated yield.



**Scheme 3.** Reagents and conditions: a) CDI,  $\text{Cl}_2\text{CH}_2$ , 40 °C, 16 h, 71%; b) i)  $\text{ICH}_3$ , acetonitrile, 60 °C, 2 h, ii)  $\text{RNH}_2$ ,  $\text{Cl}_2\text{CH}_2$ , 4 h, rt, 79–83%; c) i)  $\text{ICH}_3$ , acetonitrile, 60 °C, 2 h, ii) ethylenediamine,  $\text{Cl}_2\text{CH}_2$ , 4 h, rt, 51%; d) **23**, sodium ascorbate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{H}_2\text{O}$ –EtOH, rt, 64%.

H-20);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  171.9 (C-15), 134.9 (C-8), 126.5 (C-7), 90.6 (C-9), 81.6 (C-13), 51.4 (OMe), 47.9 (C-14), 42.7 (C-5), 42.0 (C-3), 40.7 (C-10), 38.2 (C-12), 33.2 (C-4), 32.9 (C-18), 32.8 (C-1), 28.5 (C-11), 27.3 (C-16), 24.2 (C-6), 22.4 (C-19), 21.3 (C-17), 18.7 (C-2), 16.6 (C-20); HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{21}\text{H}_{34}\text{O}_3$  [ $\text{M}$ ] $^+$ : 334.2508, found 334.2560. All data are consistent with literature [13].

#### 4.1.2. Preparation of grindelic alcohol (**4**)

A solution of 5 g of **3** (14.94 mmol) and 2.83 g (74.74 mmol) of  $\text{LiAlH}_4$  in dry THF (150 mL, 0.1 M) was refluxed during 2 h under Ar pressure. When reaction was completed the mixture was cooled and ethyl acetate was added until complete elimination of  $\text{LiAlH}_4$  residues. The crude was filtered and solids washed with 100 mL  $\text{Et}_2\text{O}$ . Water (100 mL) was added and extracted with  $\text{Et}_2\text{O}$  ( $3 \times 50$  mL). Organic layers were dried with  $\text{MgSO}_4$  and resulting crude was purified by silica gel chromatography. 4.18 g (13.64 mmol, 91% yield) of **4** was recovered as a white solid, mp 64–66 °C.  $[\alpha]_D^{20} = -131.7$  (c 1.25,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.51 (s, 1H), 3.97 (t,  $J = 11$  Hz, 1H), 3.69 (dd,  $J = 8.8$  and 3 Hz, 1H), 2.10–1.95 (m, 4H), 1.91–1.72 (m, 3H), 1.82 (s, 3H), 1.65–1.50 (m, 6H), 1.48–1.36 (m, 2H), 1.31 (s, 3H), 1.25 (m, 1H), 0.91 (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  134.5, 126.9, 91.1, 84.4, 60.0, 43.7, 42.9, 41.9, 40.7, 40.5, 33.2, 32.9, 32.5, 27.5, 26.6, 24.1, 22.2, 21.1, 18.7, 16.6; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{20}\text{H}_{34}\text{O}_2$  [ $\text{M}$ ] $^+$ : 306.2559, found 306.2548. All data are consistent with literature [13].

#### 4.1.3. Preparation of (*E*)-ethyl 2-methyl-4-((1*R*,4*a*'*S*,5*S*,8*a*'*S*)-2',5',5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)but-2-enoate (**5**)

2.32 g of **4** (7.58 mmol), 6.31 mL of  $\text{Et}_3\text{N}$  (4.6 g, 45.51 mmol) and 5.28 g of ethyl 2-(triphenyl-phosphoranylidene)propanoate (15.17 mmol) were stirred with 30 mL of dry  $\text{CH}_2\text{Cl}_2$  (0.25 M) and 6.1 mL of DMSO (0.8 mL/mmol of alcohol). When phosphorane was completely dissolved 3.62 g of  $\text{SO}_3 \cdot \text{Py}$  complex (22.75 mmol) was added. Reaction was monitored by TLC. When reaction was

completed, 20 mL of HCl (5%) was added. The layers were partitioned, and the aqueous phase was extracted with  $\text{Et}_2\text{O}$  ( $3 \times 20$  mL). Organic layers were washed with water ( $3 \times 20$  mL) and aqueous NaCl saturated solution ( $1 \times 20$  mL), dried with  $\text{MgSO}_4$ , filtered and concentrated. The crude was purified by silica gel column chromatography to give **5** (2.18 g, 5.6 mmol, 74% yield).  $[\alpha]_D^{20} = -36.1$  (c 2.81, MeOH);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  6.77 (t,  $J = 7$  Hz, 1H), 5.52 (s, 1H), 4.21 (q,  $J = 7$  Hz, 2H), 2.61 (dd,  $J = 14$  and 8 Hz, 1H), 2.46 (dd,  $J = 14$  and 8 Hz, 1H), 2.15–1.95 (m, 4H), 1.90–1.65 (m, 6H), 1.86 (s, 3H), 1.78 (s, 3H), 1.60–1.35 (m, 4H), 1.31 (t,  $J = 7$  Hz, 3H), 1.22 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.2, 138.8, 135.0, 129.3, 126.5, 90.5, 83.4, 60.4, 43.0, 42.6, 42.1, 40.7, 38.2, 33.2, 32.9, 32.8, 28.4, 27.5, 24.2, 22.4, 21.3, 18.8, 16.7, 14.3, 12.6; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{25}\text{H}_{40}\text{O}_3$  [ $\text{M}$ ] $^+$ : 388.2977, found 388.2992.

#### 4.1.4. Preparation of (*E*)-2-methyl-4-((1*R*,4*a*'*S*,5*S*,8*a*'*S*)-2',5',5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)but-2-en-1-ol (**6**)

896 mg of **5** (2.39 mmol) was dissolved in 24 mL of dry  $\text{Et}_2\text{O}$  at 0 °C. 5.26 mL of DIBAL-H solution (0.1 M in cyclohexane, 5.26 mmol) was added dropwise. When reaction was completed 5 mL of water was added and reaction was stirred for 15 min at room temperature. Reaction mixture was dried with  $\text{MgSO}_4$ , filtered through celite and purified by silica gel column chromatography to give 705 mg of **6** (2.12 mmol, 89% yield).  $[\alpha]_D^{20} = -28.2$  (c 1.06, MeOH);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.51 (s, 1H), 5.45 (td,  $J = 7$  and 2 Hz, 1H), 4.04 (s, 2H), 2.48 (dd,  $J = 14$  and 8 Hz, 1H), 2.34 (dd,  $J = 14$  and 8 Hz, 1H), 2.13–1.92 (m, 4H), 1.88–1.80 (m, 2H), 1.79 (s, 3H), 1.71–1.68 (m, 2H), 1.69 (s, 3H), 1.60–1.50 (m, 2H), 1.48–1.35 (m, 2H), 1.28–1.22 (m, 2H), 1.20 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  136.4, 135.3, 126.3, 122.9, 90.3, 83.9, 69.0, 42.6, 42.0, 41.9, 40.8, 38.0, 33.2, 32.9, 32.8, 28.4, 27.0, 24.2, 22.3, 21.2, 18.8, 16.7, 14.0; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{23}\text{H}_{38}\text{O}_2$  [ $\text{M}$ ] $^+$ : 346.2872, found 346.2872.



**Table 4**  
*In vitro* antiproliferative activity against human solid tumor cell lines.<sup>a</sup>

Comp.	Cell line				
	HBL-100 (breast)	HeLa (cervix)	SW1573 (NSCLC)	T-47D (breast)	WiDr (colon)
1	46 (±10)	60 (±31)	49 (±28)	69 (±5.5)	57 (±13)
2	>100	>100	>72 (±39)	>100	>100
3	17 (±2.4)	16 (±2.9)	18 (±1.9)	19 (±5.4)	23 (±3.9)
4	17 (±2.6)	17 (±2.5)	19 (±1.1)	16 (±1.8)	21 (±0.65)
5	17 (±4.4)	18 (±5.8)	20 (±4.5)	32 (±9.1)	37 (±0.87)
6	16 (±2.6)	16 (±2.3)	18 (±1.3)	15 (±1.7)	19 (±0.23)
7	16 (±3.8)	19 (±7.4)	18 (±3.3)	32 (±7.8)	35 (±0.69)
8	17 (±1.7)	18 (±2.5)	17 (±1.3)	17 (±2.0)	17 (±1.2)
9	16 (±2.3)	17 (±1.7)	18 (±0.64)	16 (±2.0)	19 (±0.18)
10	16 (±1.9)	17 (±1.4)	18 (±1.0)	15 (±2.5)	17 (±0.14)
11	4.6 (±3.9)	5.3 (±5.6)	11 (±1.4)	3.9 (±0.87)	12 (±0.84)
12	10 (±3.5)	30 (±6.9)	14 (±2.6)	40 (±4.5)	49 (±7.9)
13	3.7 (±0.31)	4.7 (±0.84)	3.6 (±0.71)	4.4 (±1.0)	5.1 (±0.82)
14	8.9 (±2.2)	11 (±1.6)	5.1 (±1.9)	31 (±5.8)	38 (±7.8)
15	1.9 (±0.44)	2.9 (±0.89)	1.8 (±0.51)	3.0 (±0.78)	3.1 (±1.3)
16	16 (±0.88)	18 (±2.0)	16 (±3.3)	19 (±2.3)	12 (±2.4)
17	0.95 (±0.38)	1.6 (±0.46)	1.2 (±0.54)	1.9 (±0.62)	1.8 (±0.81)
18	5.4 (±2.1)	5.7 (±2.6)	5.8 (±2.6)	5.3 (±2.5)	6.8 (±0.28)
19	8.7 (±0.63)	22 (±2.3)	5.9 (±1.2)	21 (±3.5)	37 (±8.2)
23	>100	>100	>100	>100	>100
24	14 (±3.5)	17 (±2.6)	17 (±3.3)	26 (±8.1)	15
26	17 (±1.9)	16 (±1.2)	15 (±2.4)	28 (±10)	4.8
27	16 (±5.0)	25 (±6.8)	15 (±2.3)	44 (±13)	15
28	>100	>100	>100	>100	>100
29	9.3 (±1.4)	17 (±0.82)	11 (±0.5)	17 (±1.0)	n.a.
30	2.9 (±0.49)	2.7 (±0.74)	3.0 (±0.39)	4.3 (±0.63)	1.3
32	>100	>100	>100	>100	>100
33	16 (±0.34)	18 (±1.1)	17 (±0.85)	17 (±0.88)	13
34	6.1 (±0.57)	7.8 (±2.9)	8.0 (±6.9)	4.3 (±0.98)	1.4
35	14 (±0.35)	14 (±1.6)	13 (±2.3)	16 (±2.4)	12 (±1.9)
37	>100	>100	>100	>100	>100
Cisplatin	1.9 (±0.2)	2.0 (±0.3)	3.0 (±0.4)	15 (±2.3)	26 (±5.3)
Camptothecin	0.23 (±0.05)	0.6 (±0.4)	0.25 (±0.12)	2.0 (±0.5)	1.8 (±0.7)

<sup>a</sup> Values expressed as GI<sub>50</sub> (50% growth inhibition) are given in μM and are means of two to four experiments, standard deviation is given in parentheses.

#### 4.1.5. Preparation of (E)-ethyl 4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-enoate (7)

995 mg of **4** (3.25 mmol), 2.72 mL of Et<sub>3</sub>N (1.97 g, 19.5 mmol) and 2.26 g of ethyl(triphenyl-phosphoranylidene)acetate (6.5 mmol) were stirred with 13 mL of dry CH<sub>2</sub>Cl<sub>2</sub> (0.25 M) and 2.6 mL of DMSO (0.8 mL/mmol of alcohol). When phosphorane was completely dissolved, 1.55 g of SO<sub>3</sub>·Py complex (9.74 mmol) was added. Reaction was monitored by TLC. When reaction was completed, 20 mL of HCl (5%) was added. The layers were partitioned, and the aqueous phase was extracted with Et<sub>2</sub>O (3 × 20 mL). Organic layers were washed with water (3 × 20 mL) and aqueous NaCl saturated solution (1 × 20 mL), dried with MgSO<sub>4</sub>, filtered and concentrated. Crude material was purified by silica gel column chromatography yielding 1.14 g of **7** (3.05 mmol, 94% yield). [α]<sub>D</sub><sup>20</sup> = −121.76 (c 1.71, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 6.95 (m, 1H), 5.87 (d, J = 12 Hz, 1H), 5.52 (s, 1H), 4.21 (q, J = 7 Hz, 2H), 2.60 (m, 1H), 2.47 (m, 1H), 2.21–1.98 (m, 4H), 1.88–1.79 (m, 3H), 1.77 (s, 3H), 1.72–1.38 (m, 6H), 1.31 (t, J = 7 Hz, 3H), 1.22 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 166.6, 146.1, 134.9, 126.6, 123.6, 90.6, 82.6, 60.2, 46.8, 42.6, 42.0, 40.7, 38.2, 33.2, 32.9, 32.8, 28.4, 27.5, 24.2, 22.4, 21.3, 18.8, 16.7, 14.3; HRMS-ES (m/z): calcd for C<sub>24</sub>H<sub>38</sub>O<sub>3</sub> [M]<sup>+</sup>: 374.2821, found 374.2838.

#### 4.1.6. Preparation of (E)-2-methyl-4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)but-2-en-1-ol (8)

426.2 mg of **7** (1.13 mmol) was dissolved in 12 mL of dry Et<sub>2</sub>O at 0 °C. 2.5 mL of DIBAL-H (0.1 M in cyclohexane, 2.5 mmol) was added

dropwise. When reaction was completed 3 mL of water was added and reaction was stirred for 15 min at room temperature. Reaction mixture was dried with MgSO<sub>4</sub>, filtered through Celite and purified by silica gel column chromatography to yield **8** (308.8 mg, 0.93 mmol, 82% yield). [α]<sub>D</sub><sup>20</sup> = −75.3 (c 6.22, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 5.66 (m, 2H), 5.49 (bs, 1H), 4.09 (bs, 2H), 2.44 (m, 1H), 2.32 (m, 1H), 2.11–1.92 (m, 4H), 1.88–1.70 (m, 2H), 1.76 (s, 3H), 1.70–1.60 (m, 4H), 1.58–1.45 (m, 2H), 1.44–1.20 (m, 2H), 1.18 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.80 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 135.0, 131.6, 129.4, 126.2, 90.3, 83.1, 62.4, 46.6, 42.5, 41.9, 40.6, 37.9, 33.1, 32.8, 32.7, 28.3, 27.0, 24.1, 22.3, 21.2, 18.7, 16.6; HRMS-ES (m/z): calcd for C<sub>22</sub>H<sub>36</sub>O<sub>2</sub> [M]<sup>+</sup>: 332.2715, found 332.2718.

#### 4.1.7. Preparation of ((2S,3S)-3-(((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)oxiran-2-yl)methanol (9)

In a reaction flask containing powered activated 4 Å molecular sieves (30 mg), 130 mg of **8** (0.39 mmol) was dissolved in 4 mL of dry CH<sub>2</sub>Cl<sub>2</sub> (0.1 M) and cooled at −20 °C under argon atmosphere. 138.5 μL of Ti(OPr-i)<sub>4</sub> (133.46 mg, 0.47 mmol) and 93.8 μL of (R,R)-(+)-DET (112.98 mg, 0.55 mmol) were added sequentially. The mixture was stirred at the same temperature for 20 min, and TBHP (0.14 mL, 5.5 M in isooctane, 0.78 mmol) was added slowly. After the addition, the reaction was maintained with stirring for 12 h at low temperature. Tartaric acid aqueous solution (15%w/v) was added and the stirring was continued at room temperature for 1 h. The phases were separated and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 3 mL). The combined organic phase were washed with brine, concentrated, diluted with Et<sub>2</sub>O, and treated with precooled, at 0 °C, 15%(w/v) NaOH aqueous solution. The two-phase mixture was stirred vigorously for 15 min at 0 °C. The organic phase was separated, and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 3 mL). The combined organic phase were washed with brine, dried, filtered, concentrated, and purified by silica gel column chromatography to yield **9** (96.8 mg, 0.28 mmol, 71% yield). [α]<sub>D</sub><sup>20</sup> = −97.3 (c 1.1, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 5.51 (s, 1H), 3.92 (dd, J = 12 and 2 Hz, 1H), 3.66 (dd, J = 12 and 4 Hz, 1H), 3.06 (m, 1H), 2.92 (m, 1H), 2.15–2.00 (m, 3H), 1.98–1.80 (m, 2H), 1.88–1.77 (m, 2H), 1.78 (s, 3H), 1.71–1.65 (m, 2H), 1.62–1.51 (m, 4H), 1.48–1.35 (m, 1H), 1.30 (s, 3H), 1.28–1.18 (m, 1H), 0.90 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 135.0, 126.5, 90.6, 82.3, 61.7, 58.4, 53.4, 45.4, 42.6, 42.0, 40.7, 38.5, 33.2, 32.9, 32.8, 28.3, 28.0, 24.2, 22.4, 21.2, 18.8, 16.7; HRMS-ES (m/z): calcd for C<sub>22</sub>H<sub>36</sub>O<sub>3</sub> [M]<sup>+</sup>: 348.2664, found 348.2672.

#### 4.1.8. Preparation of ((2R,3R)-3-(((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)oxiran-2-yl)methanol (10)

The procedure used above to obtain **9** was applied again to **8** (64 mg, 0.19 mmol), using the (S,S)-(−)-DET instead of (R,R)-(+)-DET, to yield **10** (57.0 mg, 0.16 mmol, 85% yield). [α]<sub>D</sub><sup>20</sup> = −137.6 (c 2.82, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 5.49 (s, 1H), 3.89 (dd, J = 12 and 2 Hz, 1H), 3.63 (dd, J = 12 and 4 Hz, 1H), 3.10 (m, 1H), 2.90 (m, 1H), 2.19–1.95 (m, 5H), 1.86–1.73 (m, 2H), 1.74 (s, 3H), 1.72–1.60 (m, 2H), 1.58–1.49 (m, 2H), 1.48–1.35 (m, 2H), 1.32 (s, 3H), 1.28–1.17 (m, 2H), 0.91 (s, 3H), 0.88 (s, 3H), 0.82 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 135.1, 126.3, 90.4, 82.2, 61.8, 58.1, 53.8, 45.8, 42.8, 42.0, 40.7, 39.2, 33.2, 33.0, 32.7, 28.4, 27.4, 24.2, 22.4, 21.3, 18.8, 16.7; HRMS-ES (m/z): calcd for C<sub>22</sub>H<sub>36</sub>O<sub>3</sub> [M]<sup>+</sup>: 348.2664, found 348.2672.

#### 4.1.9. General procedure for Ugi reaction. Preparation of 2-(N-benzyl-2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)acetamide)-N-tert-butyl-2-methylpropanamide (11)

A solution of acetone (0.030 mL, 0.41 mmol), and benzylamine (0.038 mL, 0.35 mmol) in methanol (0.7 mL, 0.5 M) were stirred for

3 h. After that, grindelic acid (107.1 mg, 0.35 mmol) and *tert*-butylisocyanide (0.044 mL, 0.38 mmol) were added. Reaction mixture was stirred for 24 h. The solvent was removed under reduced pressure and the crude reaction mixture was purified by flash chromatography on silica gel to afford the desired product **11** (111 mg, 59% yield) as a white solid: mp 72–74 °C.  $[\alpha]_D^{20} = -137.6$  (c 2.82, MeOH);  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.37 (m, 2H, H-3' and H-5'), 7.29 (m, 3H, H-2', H-4' and H-6'), 5.59 (s, 1H, N-H), 5.46 (s, 1H, H-7), 5.07 (d,  $J = 14.4$  Hz, 1H, H-7'A), 4.45 (d,  $J = 14.4$  Hz, 1H, H-7'B), 2.89 (d,  $J = 11.6$  Hz, 1H, H-14A), 2.56 (d,  $J = 11.6$  Hz, 1H, H-14B), 2.07 (m, 1H, H-11A), 2.04 (m, 3H, H-6A and H-12), 1.86 (m, 1H, H-6B), 1.73 (m, 1H, H-11B), 1.62 (m, 1H, H-5), 1.56 (s, 3H, H-17), 1.55 (m, 2H, H-1), 1.52 (m, 2H, H-2), 1.40 (s, 3H, H-16), 1.38 (s, 6H, H-3'' and H-4''), 1.36 (s, 9H, H-2''', H-3''' and H-4'''), 1.18 (m, 1H, H-3A), 1.13 (m, 1H, H-3B), 0.92 (s, 3H, H-19), 0.88 (s, 3H, H-18), 0.81 (s, 3H, H-20);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  174.0 (C-1''), 171.9 (C-15), 139.0 (C-1'), 135.0 (C-8), 128.8 (2C, C-3 and C-5'), 127.1 (C-7), 126.4 (C-4'), 125.7 (2C, C-2 and C-6'), 90.4 (C-9), 83.1 (C-13), 63.1 (C-2''), 50.7 (C-1'''), 48.0 (C-14), 47.6 (C-7'), 43.0 (C-5), 42.0 (C-3), 40.6 (C-10), 39.7 (C-12), 33.2 (C-4), 33.1 (C-18), 32.6 (C-1), 28.6 (3C, C-2''', C-3''' and C-4'''), 28.3 (C-11), 27.7 (C-16), 24.4 (C-6), 24.3 (2C, C-3'' and C-4''), 22.3 (C-19), 21.2 (C-17), 18.7 (C-2), 16.6 (C-20); HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{35}\text{H}_{54}\text{N}_2\text{O}_3$   $[\text{M}]^+$ : 550.3734, found 550.3722.

**4.1.10. Preparation of 2-(*N*-benzyl-2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-2-methyl-*N*-(2,3,3-trimethylbutan-2-yl)propanamide (**12**)**

This compound was prepared by General procedure for Ugi reaction using benzylamine and 1,1,3,3-tetramethylbutylisocyanide to obtain 444 mg of **12** (75% yield) as a clear oil color less oil.  $[\alpha]_D^{20} = -66.73$  (c 1.2,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.38 (m, 2H), 7.30 (m, 3H), 5.63 (s, 1H), 5.45 (s, 1H), 5.00 (d,  $J = 18$  Hz, 1H), 4.48 (d,  $J = 18$  Hz, 1H), 2.89 (d,  $J = 15$  Hz, 1H), 2.57 (d,  $J = 15$  Hz, 1H), 2.15–2.00 (m, 5H), 1.91–1.82 (m, 1H), 1.77–1.65 (m, 2H), 1.61 (s, 3H), 1.57 (m, 1H), 1.54–1.49 (m, 2H), 1.45 (s, 6H), 1.40 (s, 3H), 1.38 (s, 3H), 1.37 (s, 3H), 1.30–1.25 (m, 1H), 1.22–1.14 (m, 1H), 1.01 (s, 9H), 0.91 (s, 3H), 0.88 (s, 3H), 0.81 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.4, 171.9, 138.9, 135.1, 128.8 (2C), 127.1, 126.4, 125.8 (2C), 90.3, 83.2, 63.1, 54.9, 53.0, 48.0, 47.6, 43.0, 42.0, 40.6, 39.5, 33.2, 33.1, 32.6, 31.6 (3C), 31.5, 28.5, 28.4 (2C), 27.7, 24.4, 24.2, 22.3, 21.2, 18.7, 16.6; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{38}\text{H}_{60}\text{N}_2\text{O}_3$   $[\text{M}]^+$ : 606.4760, found 606.4756.

**4.1.11. Preparation of 2-(*N*-benzyl-2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-*N*-cyclohexyl-2-methylpropanamide (**13**)**

This compound was prepared by General procedure for Ugi reaction using benzylamine and cyclohexyl isocyanide to obtain 378 mg of **13** (71% yield) as a white solid: mp 81–83 °C.  $[\alpha]_D^{20} = -91.08$  (c 1.0,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.36 (m, 4H), 7.26 (m, 1H), 5.55 (d,  $J = 8$  Hz, 1H), 5.48 (s, 1H), 5.10 (d,  $J = 18$  Hz, 1H), 4.43 (d,  $J = 18$  Hz, 1H), 3.76 (m, 1H), 2.84 (d,  $J = 14$  Hz, 1H), 2.51 (d,  $J = 14$  Hz, 1H), 2.09–1.81 (m, 7H), 1.75–1.60 (m, 10H), 1.54 (bs, 3H), 1.45–1.30 (m, 3H), 1.40 (s, 6H), 1.37 (s, 3H), 1.28–1.05 (m, 5H), 0.91 (s, 3H), 0.88 (s, 3H), 0.80 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  174.0, 172.0, 139.1, 135.0, 128.7 (2C), 127.0, 126.4, 125.7 (2C), 90.4, 83.0, 62.5, 48.2, 47.8, 47.4, 43.1, 42.1, 40.6, 39.7, 33.2 (2C), 33.0, 32.6, 28.2, 27.8, 25.7, 24.9 (2C), 24.5 (2C), 24.3, 24.2, 22.3, 21.2, 18.7, 16.6; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{37}\text{H}_{56}\text{N}_2\text{O}_3$   $[\text{M}]^+$ : 576.4291, found 576.4288.

**4.1.12. Preparation of 2-(*N*-benzyl-2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-*N*-(2,6-dimethylphenyl)-2-methylpropanamide (**14**)**

This compound was prepared by General procedure for Ugi reaction using benzylamine and 2,6-dimethylphenyl isocyanide to

obtain 350 mg of **14** (63% yield) as a white solid: mp 181–183 °C.  $[\alpha]_D^{20} = -74.63$  (c 1.023,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.29 (m, 5H), 7.00 (m, 3H), 5.41 (bs, 1H), 5.37 (s, 1H), 5.07 (d,  $J = 19$  Hz, 1H), 4.54 (d,  $J = 19$  Hz, 1H), 2.89 (d,  $J = 15$  Hz, 1H), 2.78 (d,  $J = 15$  Hz, 1H), 2.56 (d,  $J = 15$  Hz, 1H), 2.45 (d,  $J = 15$  Hz, 1H), 2.28 (bs, 6H), 2.15–1.90 (m, 4H), 1.59 (s, 3H), 1.54 (s, 3H), 1.49–1.18 (10H), 1.37 (s, 3H), 0.82 (s, 3H), 0.81 (s, 3H), 0.78 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  175.1, 172.1, 138.8, 134.9, 128.7 (2C), 128.5 (2C), 127.8 (2C), 127.2, 127.0, 126.6, 125.7 (2C), 82.9, 62.5, 47.6, 47.5, 43.8, 43.2, 42.1, 40.5, 39.7, 39.6, 33.2 (2C), 32.6, 28.2, 28.1, 27.7, 24.5, 24.3 (2C), 22.2, 21.3, 19.5, 18.7, 16.6; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{39}\text{H}_{54}\text{N}_2\text{O}_3$   $[\text{M}]^+$ : 598.4134, found 598.4149.

**4.1.13. Preparation of 2-(*N*-benzyl-2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-*N*-butyl-2-methylpropanamide (**15**)**

This compound was prepared by General procedure for Ugi reaction using benzylamine and butyl isocyanide to obtain 438 mg of **15** (79% yield) as a white solid: mp 70–72 °C.  $[\alpha]_D^{20} = -78.53$  (c 1.34,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.35 (m, 5H), 5.77 (bs, 1H), 5.51 (s, 1H), 5.16 (d,  $J = 19$  Hz, 1H), 4.41 (d,  $J = 19$  Hz, 1H), 3.32 (m, 1H), 3.23 (m, 1H), 2.83 (d,  $J = 14$  Hz, 1H), 2.49 (d,  $J = 14$  Hz, 1H), 2.23–2.00 (m, 5H), 2.00–1.97 (m, 2H), 1.92–1.80 (m, 4H), 1.80–1.60 (m, 2H), 1.60–1.30 (m, 4H), 1.56 (bs, 3H), 1.42 (bs, 3H), 1.41 (bs, 3H), 1.36 (s, 3H), 0.93 (t,  $J = 6$  Hz, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.81 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  175.1, 172.1, 138.9, 134.9, 128.7 (2C), 127.0, 126.6, 125.7 (2C), 90.5, 82.9, 62.4, 47.7, 47.6, 43.3, 42.1, 40.6, 39.8, 39.7, 33.2 (2C), 32.5, 31.8, 28.1, 27.7, 24.5, 24.4, 24.3, 22.2, 21.3, 20.2, 18.7, 16.6, 13.8; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{35}\text{H}_{54}\text{N}_2\text{O}_3$   $[\text{M}]^+$ : 550.4134, found 550.4132.

**4.1.14. Preparation of 2-(*N*-benzyl-2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)acetamido)-2-methyl-*N*-(2-morpholinoethyl)propanamide (**16**)**

This compound was prepared by General procedure for Ugi reaction using benzylamine and 2-morpholinoethyl isocyanide to obtain 106.8 mg of **16** (79% yield) as a white solid: mp 164–166 °C.  $[\alpha]_D^{20} = -87.98$  (c 1.32,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.27 (m, 4H), 7.18 (m, 1H), 6.20 (bs, 1H), 5.41 (s, 1H), 5.04 (d,  $J = 19$  Hz, 1H), 4.29 (d,  $J = 19$  Hz, 1H), 3.65 (m, 4H), 3.30 (m, 2H), 2.73 (d,  $J = 14$  Hz, 1H), 2.50–2.41 (m, 6H), 2.38 (d,  $J = 14$  Hz, 1H), 2.10–1.90 (m, 4H), 1.85–1.50 (m, 4H), 1.47 (bs, 3H), 1.45–1.09 (m, 4H), 1.35 (bs, 3H), 1.28 (bs, 3H), 1.25 (s, 3H), 1.22–1.03 (m, 2H), 0.83 (s, 3H), 0.80 (s, 3H), 0.73 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  175.4, 172.1, 139.1, 134.9, 128.7 (2C), 126.9, 126.6, 125.7 (2C), 90.4, 82.9, 67.1, 62.3, 56.9, 53.3 (3C), 47.5, 47.2, 43.1, 42.2, 41.9, 40.5, 39.7, 36.1, 33.2 (2C), 28.1, 27.8, 24.8, 24.3, 23.4, 22.2, 21.3, 18.7, 16.6; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{37}\text{H}_{58}\text{N}_3\text{O}_4$   $[\text{M} + \text{H}]^+$ : 608.4427, found 608.4443.

**4.1.15. Preparation of *N*-benzyl-2-methyl-2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)-*N*-phenylacetamido propanamide (**17**)**

This compound was prepared by General procedure for Ugi reaction using benzylamine and benzylisocyanide to obtain 528 mg of **17** (97% yield) as a white solid: mp 76–78 °C.  $[\alpha]_D^{20} = -72.91$  (c 1.18,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.25 (m, 10H), 6.94 (m, 1H), 5.41 (s, 1H), 5.04 (d,  $J = 19$  Hz, 1H), 4.50 (dd,  $J = 19$  and 6 Hz, 1H), 4.34 (m, 2H), 2.76 (d,  $J = 14$  Hz, 1H), 2.41 (d,  $J = 19$  Hz, 1H), 2.15–1.59 (m, 6H), 1.58–1.10 (m, 6H), 1.47 (bs, 3H), 1.37 (bs, 3H), 1.36 (bs, 3H), 1.22 (s, 3H), 1.00 (m, 1H), 0.81 (s, 3H), 0.75 (s, 3H), 0.73 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  175.1, 172.1, 138.8 (2C), 134.9, 128.7 (2C), 128.5 (2C), 127.8 (2C), 127.2, 127.0, 126.6, 125.7 (2C), 90.4, 82.9, 62.5, 47.6, 47.5, 43.8, 43.2, 42.1, 40.5, 39.7, 33.2 (2C), 32.6, 28.2, 27.7, 24.5, 24.3

(2C), 22.2, 21.3, 18.7, 16.6; HRMS-ES ( $m/z$ ): calcd for  $C_{38}H_{52}N_2O_3$   $[M]^+$ : 584.3978, found 584.3956.

**4.1.16. Preparation of *N*-tert-butyl-2-methyl-2-(2-((1*R*,4*a*'*S*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)-*N*-phenylacetamido)propanamide (**18**)**

This compound was prepared by General procedure for Ugi reaction using aniline and tert-butyl-isocyanide to obtain 389.4 mg of **18** (78% yield) as a white solid: mp 74–76 °C.  $[\alpha]_D^{20} = -65.36$  (c 11.37,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  7.36 (m, 3H), 7.21 (m, 2H), 5.69 (s, 1H), 5.38 (s, 1H), 2.45 (d,  $J = 15$  Hz, 1H), 2.24 (d,  $J = 15$  Hz, 1H), 2.15 (m, 1H), 1.99 (m, 3H), 1.82–1.54 (m, 4H), 1.53 (bs, 3H), 1.51–1.42 (m, 3H), 1.38 (s, 9H), 1.33 (s, 3H), 1.32 (s, 3H), 1.22 (s, 3H), 1.20–1.08 (m, 2H), 0.87 (s, 3H), 0.81 (s, 3H), 0.77 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  174.0, 170.8, 139.9, 135.2, 130.6, 130.2, 129.4 (2C), 128.4, 126.0, 123.9, 90.0, 83.5, 63.0, 50.8, 48.1, 42.8, 42.0, 40.7, 38.8, 33.0, 32.9, 28.7, 28.6 (3C), 27.6, 25.9, 24.8, 24.1, 22.4, 21.2, 18.7, 16.7; HRMS-ES ( $m/z$ ): calcd for  $C_{34}H_{52}N_2O_3$   $[M]^+$ : 536.3978, found 536.3961.

**4.1.17. Preparation of *N*-(2,6-dimethylphenyl)-2-methyl-2-(2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)-*N*-phenylacetamido)propanamide (**19**)**

This compound was prepared by General procedure for Ugi reaction using aniline and 2,6-dimethylphenyl isocyanide to obtain 140 mg of **19** (26% yield) as a white solid: mp 90–92 °C.  $[\alpha]_D^{20} = -62.58$  (c 10.47,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  7.41 (m, 3H), 7.30 (m, 3H), 7.20 (s, 1H), 7.10 (m, 2H), 5.40 (s, 1H), 2.49 (d,  $J = 18$  Hz, 1H), 2.32 (bs, 6H), 2.30–2.15 (m, 4H), 2.12–1.89 (m, 4H), 1.80–1.58 (m, 6H), 1.53 (bs, 6H), 1.48 (bs, 3H), 1.32 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  172.9, 171.0, 139.9, 139.7, 135.3, 130.7, 130.4, 129.5 (2C), 128.6, 128.1 (2C), 126.9, 126.0, 124.3, 118.0, 90.0, 83.5, 63.0, 47.9, 42.8, 41.9, 40.7, 38.7, 33.2, 33.0, 32.9, 28.7, 27.6, 26.1, 25.5, 24.1, 22.4, 21.3, 18.7, 18.5 (2C), 16.7; HRMS-ES ( $m/z$ ): calcd for  $C_{38}H_{52}N_2O_3$   $[M]^+$ : 584.3978, found 584.3971.

**4.1.18. Preparation of *N*-butyl-2-methyl-2-(2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)-*N*-phenylacetamido)propanamide (**20**)**

This compound was prepared by General procedure for Ugi reaction using aniline and butyl isocyanide to obtain 115 mg of **20** (23% yield) as a white solid: mp 187–189 °C.  $[\alpha]_D^{20} = -52.86$  (c 1.17,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  7.38 (m, 3H), 7.27 (m, 2H), 5.87 (t,  $J = 6$  Hz, 1H), 5.41 (s, 1H), 3.32 (m, 2H), 2.47 (d,  $J = 15$  Hz, 1H), 2.26 (d,  $J = 15$  Hz, 1H), 2.15 (m, 1H), 2.05–1.90 (m, 3H), 1.85–1.72 (m, 1H), 1.70–1.60 (m, 4H), 1.60–1.45 (m, 4H), 1.52 (bs, 3H), 1.45–1.25 (m, 3H), 1.35 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H), 1.15 (m, 1H), 0.95 (t,  $J = 7$  Hz, 3H), 0.89 (s, 3H), 0.84 (s, 3H), 0.79 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  174.9, 171.0, 140.0, 135.2, 130.7, 130.3, 129.4 (2C), 128.4, 126.0, 90.1, 83.5, 62.5, 47.9, 42.9, 42.0, 40.7, 39.7, 38.9, 33.1, 33.0, 32.9, 31.7, 28.6, 27.6, 25.9, 25.1, 24.1, 22.4, 21.3, 20.2, 18.7, 16.7, 13.8; HRMS-ES ( $m/z$ ): calcd for  $C_{34}H_{52}N_2O_3$   $[M]^+$ : 536.3978, found 536.3962.

**4.1.19. Preparation of *N*-benzyl-2-methyl-2-(2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)-*N*-phenylacetamido)propanamide (**21**)**

This compound was prepared by General procedure for Ugi reaction using aniline and benzylisocyanide to obtain 229 mg of **21** (43% yield) as a white solid: mp 194–196 °C.  $[\alpha]_D^{20} = -57.56$  (c 1.16,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  7.37 (m, 7H), 7.27 (m, 3H), 6.14 (t,  $J = 6$  Hz, 1H), 5.41 (s, 1H), 4.60 (dd,  $J = 15$  and 6 Hz, 1H), 4.49 (dd,  $J = 15$  and 6 Hz, 1H), 2.47 (d,  $J = 15$  Hz, 1H), 2.26 (d,  $J = 15$  Hz, 1H), 2.20–2.08 (m, 1H), 2.07–1.81 (m, 3H), 1.75–1.55 (m, 5H), 1.53 (bs, 3H), 1.51–1.42

(m, 4H), 1.37 (s, 6H), 1.30 (s, 3H), 1.15 (m, 1H), 0.89 (s, 3H), 0.82 (s, 3H), 0.79 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  174.9, 171.1, 139.8, 138.7, 135.2, 130.7, 130.4, 129.5 (2C), 128.6 (2C), 128.5, 127.8 (2C), 127.3, 126.0, 90.1, 83.5, 62.6, 47.9, 43.9, 42.9, 42.0, 40.6, 38.8, 33.2, 33.0, 32.9, 28.7, 27.6, 25.7, 25.2, 24.1, 22.5, 21.3, 18.7, 16.7; HRMS-ES ( $m/z$ ): calcd for  $C_{37}H_{51}N_2O_3$   $[M + H]^+$ : 571.3900, found 571.3872.

**4.1.20. Preparation of *N*-cyclohexyl-2-methyl-2-(2-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)-*N*-phenylacetamido)propanamide (**22**)**

This compound was prepared by General procedure for Ugi reaction using aniline and cyclohexyl isocyanide to obtain 481 mg of **22** (92% yield) as a white solid: mp 222–224 °C.  $[\alpha]_D^{20} = -60.91$  (c 1.33,  $CHCl_3$ );  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  7.38 (m, 3H), 7.23 (m, 2H), 5.68 (d,  $J = 8$  Hz, 1H), 5.40 (s, 1H), 3.79 (m, 1H), 2.45 (d,  $J = 15$  Hz, 1H), 2.25 (d,  $J = 15$  Hz, 1H), 2.12–1.98 (m, 1H), 1.95–1.78 (m, 5H), 1.75–1.50 (m, 9H), 1.53 (bs, 3H), 1.42–1.30 (m, 3H), 1.33 (s, 3H), 1.31 (s, 3H), 1.28 (s, 3H), 1.27–1.02 (m, 5H), 0.87 (s, 3H), 0.82 (s, 3H), 0.77 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  173.9, 170.9, 140.0, 135.2, 130.6, 130.3, 129.4 (2C), 128.4, 125.9, 90.0, 83.5, 62.6, 48.4, 48.0, 42.8, 42.0, 40.6, 38.7, 33.2, 33.0 (2C), 32.9, 29.7, 28.7, 27.6, 25.8, 25.7, 25.1, 24.9 (2C), 24.1, 22.47, 21.2, 18.7, 16.7; HRMS-ES ( $m/z$ ): calcd for  $C_{36}H_{54}N_2O_3$   $[M]^+$ : 562.4134; found 562.4127.

**4.1.21. Preparation of (1*R*,4*a*'*S*,5*S*,8*a*'*S*)-5-(2-azidoethyl)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene] (**23**)**

Alcohol **4** (2 g, 6.53 mmol) was dissolved in dry  $CH_2Cl_2$  (65 mL) and the solution was cooled to 0 °C. Triethylamine (1.8 mL, 13.06 mmol) was added via syringe followed by methanesulfonyl chloride (758  $\mu$ L, 9.78 mmol) in one portion. After 4.5 h, the reaction mixture was diluted with  $CH_2Cl_2$  (20 mL), washed with  $H_2O$  (30 mL) and brine (30 mL), dried with  $MgSO_4$ , filtered, and evaporated under reduced pressure to leave a yellow residue. The crude residue was purified by flash-chromatography column on silica gel, using *n*-hexane/ethyl acetate as the eluent. The mesylate obtained (2.26 g, 5.8 mmol, 90% yield) was dissolved in dry DMF (37 mL, 0.16 M). Sodium azide (764 mg, 11.7 mmol) was added and the mixture was stirred at 40 °C. After 24 h, the reaction mixture was diluted with  $Et_2O$  (40 mL), washed with  $H_2O$  (3  $\times$  20 mL) and brine (30 mL), dried with  $MgSO_4$ , filtered, and concentrated under reduced pressure. The crude oil was purified by column chromatography on silica gel, using *n*-hexane/ethyl acetate as the eluent, to afford **23** as an oil (1.55 g, 80% yield).  $[\alpha]_D^{20} = -140.8$  (c 1.15,  $CHCl_3$ ); IR  $\nu_{max}$ ,  $cm^{-1}$  (KBr): 2968, 2924, 2870, 2098 (azide), 1460, 1377, 1315, 1267, 1003, 874, 677;  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  5.50 (s, 1H, H-7), 3.42 (m, 1H, H-15A), 3.32 (m, 1H, H-15B), 2.07 (m, 1H, H-11A), 2.05 (m, 1H, H-6A), 1.98 (m, 1H, H-12A), 1.97 (m, 1H, H-14A), 1.86 (m, 1H, H-14B), 1.83 (m, 1H, H-6B), 1.81 (m, 1H, H-11B), 1.77 (m, 1H, H-12B), 1.76 (s, 3H, H-17), 1.69 (m, 1H, H-5), 1.65 (m, 1H, H-1A), 1.57 (m, 2H, H-2), 1.41 (m, 1H, H-1B), 1.39 (m, 1H, H-3A), 1.23 (s, 3H, H-16), 1.22 (m, 1H, H-3B), 0.91 (s, 3H, H-19), 0.89 (s, 3H, H-18), 0.82 (s, 3H, H-20);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  135.0 (C-8), 126.4 (C-7), 90.4 (C-9), 81.5 (C-13), 48.2 (C-15), 42.6 (C-5), 42.1 (C-14), 42.0 (C-3), 40.7 (C-10), 39.5 (C-12), 33.2 (C-4), 33.0 (C-18), 32.8 (C-1), 28.2 (C-11), 26.9 (C-16), 24.2 (C-6), 22.3 (C-19), 21.2 (C-17), 18.8 (C-2), 16.8 (C-20); HRMS-ES ( $m/z$ ): calcd for  $C_{20}H_{34}N_3O$   $[M + H]^+$ : 332.2702, found 332.2709.

**4.1.22. Preparation of 3-((1*R*,5*S*,8*a*'*S*)-2',5,5',5',8*a*'-pentamethyl-4,4*a*',5,5',6',7',8',8*a*'-octahydro-3*H*,4'*H*-spiro[furan-2,1'-naphthalene]-5-yl)propanal (**24**)**

To an oxalyl chloride solution (60  $\mu$ L, 0.622 mmol) in  $CH_2Cl_2$  (8 mL) at –78 °C was added dropwise DMSO (90  $\mu$ L, 1.24 mmol), and the resulting mixture was stirred for 15 min. A solution of **4** (150.5 mg, 0.49 mmol) in  $CH_2Cl_2$  (2 mL) was added dropwise



at  $-78\text{ }^{\circ}\text{C}$ . The mixture was stirred for 30 min. Triethylamine (0.7 mL, 4.9 mmol) was added dropwise and the resultant mixture was gradually warmed to room temperature and stirred for 7 h. The mixture was quenched with water (10 mL) and  $\text{CH}_2\text{Cl}_2$  (10 mL). The organic layer was washed with brine, dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuum. Purification of the residue by silica gel column chromatography (*n*-hexane/ethyl acetate, 85:15) gave the aldehyde **24** (148 mg, 99%).  $[\alpha]_{\text{D}}^{25} = -72.14$  (c 0.14,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  9.82 (s, 1H), 5.52 (s, 1H), 2.80 (dd,  $J = 13$  and 2 Hz, 1H), 2.60 (dd,  $J = 13$  and 2 Hz, 1H), 2.08 (m, 4H), 1.87 (m, 3H), 1.76 (bs, 3H), 1.57 (m, 5H), 1.33 (s, 3H), 1.22 (m, 1H), 0.91 (s, 3H), 0.88 (s, 3H), 0.83 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  202.8, 134.6, 126.8, 80.9, 57.1, 42.7, 42.0, 40.7, 39.4, 33.2, 33.0, 32.8, 28.3, 28.1, 24.2, 22.3, 21.3, 18.8, 18.7, 16.7; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{20}\text{H}_{32}\text{O}_2$   $[\text{M}]^+$ : 304.24022, found 304.2412.

#### 4.1.23. Preparation of (1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-5-(prop-2-ynyl)-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene] (**26**)

Aldehyde **24** (148 mg, 0.48 mmol) was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (5 mL, 0.1 M) at  $0\text{ }^{\circ}\text{C}$  under argon atmosphere. Triphenylphosphine (514 mg, 1.96 mmol), triethylamine (0.3 mL, 1.96 mmol) and  $\text{CBr}_4$  (325 mg, 0.98 mmol) were added. The mixture was stirred for 15 min and the work-up was carried out by addition of  $\text{NaHCO}_3$  saturated solution. The product was extracted with  $\text{Et}_2\text{O}$  ( $3 \times 25$  mL), washed with brine, dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuum. The dibromoolefin **25** obtained was used without purification in the next reaction. The crude material was dissolved in THF (5 mL, 0.1 M) and cooled at  $-78\text{ }^{\circ}\text{C}$  under argon with stirring. A solution of *n*-BuLi (0.36 mL, 2.7 M in heptane) was added using triphenylmethane as indicator. After 30 min a  $\text{NH}_4\text{Cl}$  saturated solution was added and the product was extracted with  $\text{Et}_2\text{O}$  ( $3 \times 25$  mL). The organic layer was washed with brine, dried over  $\text{MgSO}_4$ , filtered and concentrated in vacuum. The residue was purified by column chromatography on silica gel, using a mixture of *n*-hexane-ethyl acetate as eluent, to afford **26** as a clear oil (78 mg, 53% total yield).  $[\alpha]_{\text{D}}^{25} = -77.5$  (c 0.48,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.53 (s, 1H), 2.57 (dd,  $J = 8$  and 2 Hz, 1H), 2.71 (t,  $J = 8$  and 2 Hz, 1H), 2.19 (m, 3H), 2.00 (s, 1H), 1.91–1.78 (m, 2H), 1.79 (bs, 3H), 1.75–1.62 (m, 1H), 1.58–1.45 (m, 2H), 1.41 (s, 3H), 1.32–1.23 (m, 2H), 0.92 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.84 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  134.9, 126.6, 91.2, 82.9, 82.5, 69.3, 42.6, 42.0, 40.9, 38.1, 33.4, 33.2, 32.9, 32.8, 28.3, 26.8, 24.2, 22.4, 21.3, 18.8, 16.7; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{21}\text{H}_{32}\text{O}$   $[\text{M}]^+$ : 300.2453, found 300.2453.

#### 4.1.24. General procedure for Huisgen reaction. Preparation of (4-decyl)-1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazole (**27**)

The azide **23** (53.7 mg, 0.16 mmol) and 1-dodecyne (35  $\mu\text{L}$ , 0.16 mmol) were suspended in a 1:1 mixture of water and ethanol (0.1 M). Sodium ascorbate (9.6 mg, 0.05 mmol) and copper(II) sulfate pentahydrate (4 mg, 0.02 mmol) were added. The resulting mixture was stirred at room temperature for 24 h. Then, the reaction was diluted with water, extracted with ethyl acetate (10 mL  $\times$  3), dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuum. The residue was purified by column chromatography on silica gel using a mixture of *n*-hexane-ethyl acetate as eluent, to obtain the pure product **27** (38.6 mg, yield 48%) as a clear oil.  $[\alpha]_{\text{D}}^{25} = -64.35$  (c 0.20,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.32 (s, 1H), 5.51 (s, 1H), 4.46 (t,  $J = 8$  Hz, 2H), 2.71 (t,  $J = 8$  Hz, 2H), 2.19 (m, 3H), 2.15–1.78 (m, 5H), 1.74 (bs, 3H), 1.71–1.49 (m, 5H), 1.45–1.08 (m, 18H), 1.28 (s, 3H), 0.92 (t,  $J = 7$  Hz, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.82 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  148.3, 134.9, 126.6, 120.6, 90.6, 81.3, 68.0, 47.0, 43.7, 42.7, 42.1, 40.7, 39.7, 33.2, 33.0, 32.8, 31.9,

29.6, 29.5 (2C), 29.3, 28.1, 26.9, 25.7, 24.3, 22.7, 22.2, 21.3, 18.8, 18.4, 16.7, 14.1; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{32}\text{H}_{55}\text{N}_3\text{O}$   $[\text{M}]^+$ : 497.4345, found 497.4329.

#### 4.1.25. Preparation of 4-(3-ethynylphenyl)-1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazole (**28**)

This compound was prepared by General procedure for Huisgen reaction using 50 mg of azide **23** (0.15 mmol) and 9.5 mg of 1,3-diethylbenzene (0.075 mmol) to obtain 54 mg of **28** (78% yield) as a white solid: mp 129–131  $^{\circ}\text{C}$ .  $[\alpha]_{\text{D}}^{20} = -175.6$  (c 1.57,  $\text{CHCl}_3$ ); IR  $\nu_{\text{max}}$ ,  $\text{cm}^{-1}$  (KBr): 3311 ( $\text{C}_{\text{sp}}\text{-H}$ ), 3130 ( $=\text{C-H}$ ), 2966, 2916, 2866, 2114 ( $\text{C}_{\text{sp}}\text{-C}_{\text{sp}}$ ), 1479, 1373, 1232, 1001, 804, 656, 619;  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.92 (s, 1H, H-5'), 7.86 (m, 2H, H-9' and H-11'), 7.46 (d,  $J = 6.3$  Hz, 1H, H-7'), 7.39 (t,  $J = 7.6$  Hz, 1H, H-10'), 5.50 (s, 1H, H-7), 4.57 (t,  $J = 7.6$  Hz, 2H, H-15), 3.12 (s, 1H, H-13'), 2.25 (t,  $J = 7.6$  Hz, 2H, H-14), 2.01 (m, 1H, H-6A), 1.99 (m, 1H, H-11A), 1.98 (m, 1H, H-12A), 1.83 (m, 2H, H-11B and H-12B), 1.78 (m, 1H, H-6B), 1.74 (s, 3H, H-17), 1.69 (m, 1H, H-5), 1.63 (m, 1H, H-1A), 1.56 (m, 2H, H-2), 1.44 (m, 1H, H-1B), 1.39 (m, 1H, H-3A), 1.30 (s, 3H, H-16), 1.22 (m, 1H, H-3B), 0.91 (s, 3H, H-19), 0.87 (s, 3H, H-18), 0.82 (s, 3H, H-20);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  146.6 (C-4'), 134.8 (C-8), 131.5 (C-5'), 131.2 (C-6'), 129.2 (C-9'), 128.8 (C-7'), 126.7 (C-7), 126.0 (C-10'), 122.6 (C-8'), 120.2 (C-11'), 90.8 (C-9), 83.3 (C-12'), 81.4 (2-C, C-13' and C-13), 47.2 (C-15), 43.5 (C-14), 42.8 (C-5), 42.1 (C-3), 40.7 (C-10), 40.1 (C-12), 33.2 (C-4), 33.0 (C-18), 32.8 (C-1), 28.1 (C-6), 27.0 (C-16), 24.2 (C-11), 22.3 (C-19), 21.2 (C-17), 18.8 (C-2), 16.7 (C-20); HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{30}\text{H}_{39}\text{N}_3\text{O}$   $[\text{M}]^+$ : 457.3093, found 457.3088.

#### 4.1.26. Preparation of 1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-4-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)methyl)-1H-1,2,3-triazole (**29**)

This compound was prepared by General procedure for Huisgen reaction using 48.0 mg of azide **23** (0.14 mmol) and 44.3 mg of alquine **26** (0.14 mmol) to obtain 58.5 mg of **29** (64% yield) as a clear oil.  $[\alpha]_{\text{D}}^{25} = -103.33$  (c 0.12,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.39 (s, 1H), 5.51 (s, 2H), 4.44 (t,  $J = 8$  Hz, 2H), 3.00 (s, 2H), 2.18 (m, 4H), 2.06 (m, 6H), 1.98–1.73 (m, 6H), 1.74 (bs, 3H), 1.69 (s, 3H), 1.68–1.47 (m, 6H), 1.41 (m, 5H), 1.25 (s, 3H), 1.20 (m, 4H), 1.18 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.81 (s, 3H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  144.9, 135.5, 134.8, 126.6, 126.2, 122.7, 90.6, 90.5, 82.7, 81.3, 60.4, 47.0, 43.6, 42.7, 42.6, 42.1 (2C), 40.8, 40.7, 39.6, 39.5, 38.4, 33.3, 33.2, 33.1, 33.0, 32.8, 29.7, 28.3, 28.1, 27.2, 26.9, 24.3 (2C), 22.3 (2C), 21.3, 21.0, 18.8, 16.7, 14.2; HRMS-ES ( $m/z$ ): calcd for  $\text{C}_{41}\text{H}_{65}\text{N}_3\text{O}_2$   $[\text{M}]^+$ : 631.5077, found 631.5073.

#### 4.1.27. Preparation of 1,4-bis(1-(2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)butane (**30**)

This compound was prepared by General procedure for Huisgen reaction using 27.0 mg of azide **23** (0.08 mmol) and 5  $\mu\text{L}$  of 1,7-octadiyne (0.04 mmol) to obtain 22.4 mg of **30** (64% yield) as a clear oil, yield 64%.  $[\alpha]_{\text{D}}^{25} = -84.57$  (c 0.31,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.34 (s, 2H, H-5'), 5.50 (s, 2H, H-7), 4.46 (t,  $J = 7.6$  Hz, 4H, H-15), 2.74 (t,  $J = 7.14$  Hz, 4H, H-6'), 2.18 (m, 4H, H-14), 2.03 (m, 2H, H-11A), 1.95 (m, 2H, H-12A), 1.85 (m, 2H, H-11B), 1.80 (m, 8H, H-6 and H-7'), 1.79 (m, 2H, H-12B), 1.73 (s, 6H, H-17), 1.70 (m, 2H, H-5), 1.63 (m, 2H, H-1A), 1.61 (m, 4H, H-2), 1.43 (m, 2H, H-1B), 1.41 (m, 2H, H-3A), 1.26 (s, 6H, H-16), 1.21 (m, 2H, H-3B), 0.91 (s, 6H, H-19), 0.89 (s, 6H, H-18), 0.81 (s, 6H, H-20);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  147.7 (2C, C-4'), 134.8 (2C, C-8), 126.3 (2C, C-7), 120.6 (2C, C-5'), 90.8 (2C, C-9), 81.3 (2C, C-13), 47.1 (2C, C-15), 43.7 (2C, C-14), 42.7 (2C, C-5), 42.1 (2C, C-3), 40.7 (2C, C-10), 39.8 (2C, C-12), 33.2 (2C, C-

4), 33.0 (2C, C-18), 32.8 (2C, C-1), 28.5 (2C, C-6), 28.2 (2C, C-7'), 26.9 (2C, C-16), 25.2 (2C, C-6'), 24.3 (2C, C-11), 22.3 (2C, C-19), 21.3 (2C, C-17), 18.8 (2C, C-2), 16.7 (2C, C-20).

**4.1.28. Preparation of 1,3-bis(1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)benzene (**31**)**

This compound was prepared by General procedure for Huisgen reaction using 87.0 mg of azide **23** (0.26 mmol) and 17  $\mu$ L of 1,3-diethynylbenzene (0.13 mmol) to obtain 51.0 mg of **31** (51% yield) as a white solid: mp 101–103 °C.  $[\alpha]_D^{20} = -144.5$  (c 8.8, CHCl<sub>3</sub>); IR  $\nu_{\max}$ , cm<sup>-1</sup> (KBr): 3136 (=C–H), 2962, 2926, 2870, 1699, 1460, 1379, 1261, 1101, 1020, 800; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  8.28 (s, 1H), 7.91 (s, 2H), 7.82 (dd, *J* = 8 and 2 Hz, 2H), 7.49 (t, *J* = 8 Hz, 1H), 5.52 (s, 2H), 4.58 (m, 4H), 2.28 (m, 4H), 2.17–1.92 (m, 8H), 1.89–1.78 (m, 6H), 1.77 (s, 6H), 1.74–1.49 (m, 6H), 1.46–1.38 (m, 4H), 1.32 (s, 6H), 1.29–1.10 (m, 2H), 0.92 (s, 6H), 0.88 (s, 6H), 0.83 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  134.7 (2C), 131.4 (2C), 126.7 (2C), 125.2 (2C), 122.8 (2C), 120.0 (2C), 90.7 (2C), 81.3 (2C), 77.2 (2C), 47.3 (2C), 43.7 (2C), 42.7 (2C), 42.1 (2C), 40.7 (2C), 39.9 (2C), 33.2 (2C), 33.0 (2C), 32.8 (2C), 28.1 (2C), 26.9 (2C), 24.2 (2C), 22.3 (2C), 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ES (*m/z*): calcd for C<sub>50</sub>H<sub>72</sub>N<sub>6</sub>O<sub>2</sub> [M + H]<sup>+</sup>: 788.5717, found 788.5724. HRMS-ESI (*m/z*): calcd for C<sub>50</sub>H<sub>72</sub>N<sub>6</sub>O<sub>2</sub>Na [M + Na]<sup>+</sup>: 811.5614, found 811.5606.

**4.1.29. Preparation of 1,3-bis(1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzene (**32**)**

This compound was prepared by General procedure for Huisgen reaction using 100.0 mg of azide **23** (0.30 mmol) and 28.0 mg of 1,3-bis(prop-2-yn-1-yloxy)benzene (0.15 mmol) to obtain 59.0 mg of **32** (46% yield) as a white solid: mp 75–77 °C.  $[\alpha]_D^{20} = -123.4$  (c 1.08, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.67 (bs, 2H), 7.20 (t, *J* = 8 Hz, 1H), 6.63 (s, 1H), 6.62 (d, *J* = 8 Hz, 2H), 5.49 (bs, 2H), 5.19 (s, 4H), 4.51 (m, 4H), 2.21 (t, *J* = 8, 4H), 2.15–1.90 (m, 8H), 1.90–1.75 (m, 6H), 1.72 (s, 6H), 1.71–1.46 (m, 8H), 1.45–1.30 (m, 2H), 1.28 (s, 6H), 1.28–1.15 (m, 2H), 0.91 (s, 6H), 0.89 (s, 6H), 0.81 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  159.5 (2C), 144.0 (2C), 134.7, 130.0, 126.6 (2C), 122.9 (2C), 107.5 (2C), 102.2 (2C), 90.7 (2C), 81.2 (2C), 62.1 (2C), 47.4 (2C), 43.6 (2C), 42.7 (2C), 42.1 (2C), 40.7 (2C), 39.9 (2C), 33.2 (2C), 33.0 (2C), 32.7 (2C), 28.0 (2C), 26.9 (2C), 24.3 (2C), 22.3 (2C), 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ESI (*m/z*): calcd for C<sub>52</sub>H<sub>76</sub>N<sub>6</sub>O<sub>4</sub>Na [M + Na]<sup>+</sup>: 871.5825, found 871.5832.

**4.1.30. Preparation of 2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl-1H-imidazole-1-carboxylate (**33**)**

To a solution of alcohol **4** (1 g, 3.26 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (65 mL, 0.05 M) was added carbonyl diimidazole (2.64 g, 16.4 mmol). The reaction mixture was heated to 40 °C and stirred for 4.5 h. Solvent was removed and the residue subjected to a flash chromatography to provide carbonyl imidazole **33** (927 mg, yield 71%) as clear oil.  $[\alpha]_D^{20} = -117.90$  (c 15.54, CHCl<sub>3</sub>); IR  $\nu_{\max}$ , cm<sup>-1</sup> (KBr): 3124, 2970, 1747 (C=O), 1672 (C=C), 1466, 1379, 1271, 1005, 872, 758; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  8.11 (s, 1H, H-2'), 7.41 (s, 1H, H-4'), 7.05 (s, 1H, H-5'), 5.46 (bs, 1H, H-7), 4.56 (m, 2H, H-15), 2.16 (dt, *J* = 14 and 7 Hz, 1H, H-14A), 2.07 (m, 1H, H-11A), 2.03 (m, 1H, H-14B), 2.00 (m, 1H, H-12A), 1.96 (m, 1H, H-6A), 1.79 (m, 2H, H-11B and H-12B), 1.76 (s, 3H, H-17), 1.76 (m, 1H, H-6B), 1.67 (m, 1H, H-5), 1.61 (m, 2H, H-3), 1.51 (m, 2H, H-2), 1.40 (m, 2H, H-1), 1.28 (s, 3H, H-16), 0.89 (s, 3H, H-19), 0.85 (s, 3H, H-18), 0.80 (s, 3H, H-20); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  137.1 (C-2'), 134.8 (C-8), 130.5 (C-4'), 126.6 (C-7), 117.1 (C-5'), 90.6 (C-9), 81.3 (C-13), 65.9 (C-15), 42.6 (C-5), 42.0 (C-3), 41.8 (C-14), 40.7 (C-10), 40.0 (C-12), 33.2 (C-4), 33.0 (C-18), 32.7 (C-1), 27.1 (C-16), 24.2 (2C, C-11 and C-6), 22.3 (C-19), 21.3 (C-17), 18.7

(C-2), 16.7 (C-20); HRMS-ES (*m/z*): calcd for C<sub>24</sub>H<sub>37</sub>N<sub>2</sub>O<sub>3</sub> [M + H]<sup>+</sup>: 401.2799, found 401.2791.

**4.1.31. General procedure for synthesis of carbamates derivatives.**

**Preparation of 2-((1'R,4a'S,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl-3-(dimethylamino)propylcarbamate (**34**)**

A solution of the carbonyl imidazole **33** (24.2 mg, 0.06 mmol) was dissolved in acetonitrile (0.12 mL, 0.5 M). Methyl iodide (0.75 mL, 12 mmol) was added and the solution was heated to 60 °C for 2 h. The volatiles were removed by evaporation and the residue was redissolved in methylene chloride (1.5 mL, 0.04 M), 3-(dimethylamino)-1-propylamine (0.15 mL, 1.2 mmol) was added, and the resulting reaction mixture was stirred 4 h. The volatiles were removed by evaporation and the crude residue was purified by flash chromatography to give 20.7 mg of **34** (79% yield) as a clear oil.  $[\alpha]_D^{25} = -38$  (c 0.1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  5.53 (bs, 1H), 5.49 (bs, 1H), 4.16 (bs, 2H), 3.26 (m, 2H), 2.58 (m, 2H), 2.40 (bs, 6H), 2.06 (m, 6H), 1.78 (m, 1H), 1.76 (s, 3H), 1.69 (m, 4H), 1.51 (m, 2H), 1.40 (m, 2H), 1.23 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.81 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  156.9, 135.2, 126.2, 90.2, 81.9, 62.6, 56.8, 44.4, 42.6, 42.4, 42.0, 40.7, 39.2 (2C), 33.2, 32.9, 32.8, 29.7, 28.4, 27.0, 26.3, 24.2, 22.4, 21.2, 18.8, 16.7; HRMS-ESI (*m/z*): calcd for C<sub>26</sub>H<sub>46</sub>N<sub>2</sub>O<sub>3</sub>Na [M + Na]<sup>+</sup>: 457.3406, found 457.3407.

**4.1.32. Preparation of 2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl prop-2-ynylcarbamate (**35**)**

This compound was prepared by General procedure for synthesis of carbamates derivatives using 97.0 mg carbonyl imidazole **33** (0.24 mmol) and 0.35 mL of propargylamine (5.52 mmol) to obtain 78 mg of **35** (83% yield) as a white solid: mp 116–118 °C.  $[\alpha]_D^{20} = -113.7$  (c 0.918, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  5.49 (bs, 1H), 4.83 (bs, 1H), 4.20 (m, 2H), 3.98 (bs, 2H), 2.25 (m, 1H), 2.10–1.88 (m, 4H), 1.78–1.60 (m, 4H), 1.76 (s, 3H), 1.59–1.45 (m, 3H), 1.40–1.35 (m, 2H), 1.28–1.20 (m, 2H), 1.23 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.82 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  135.2, 134.2, 126.3, 90.4, 81.8, 80.8, 71.5, 63.2, 42.6, 42.3, 42.0, 40.7, 39.3, 33.2, 32.9, 32.8, 29.7, 28.4, 27.0, 24.2, 22.4, 21.2, 18.8, 16.7; HRMS-ES (*m/z*): calcd for C<sub>24</sub>H<sub>37</sub>NO<sub>3</sub> [M]<sup>+</sup>: 387.2773, found 387.2761.

**4.1.33. Preparation of bis(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl) ethane-1,2-diyl dicarbamate (**36**)**

This compound was prepared by General procedure for synthesis of carbamates derivatives using 100.0 mg carbonyl imidazole **33** (0.25 mmol) and 0.38 mL of ethylenediamine (5.75 mmol) to obtain 46 mg of **36** (51% yield) as a white solid: mp 170–172 °C.  $[\alpha]_D^{20} = -126.7$  (c 1.12, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  5.50 (bs, 2H), 4.99 (bs, 2H), 4.17 (m, 4H), 3.30 (bs, 4H), 2.09–1.80 (m, 8H), 1.79–1.62 (m, 2H), 1.77 (s, 6H), 1.60–1.52 (m, 4H), 1.51–1.38 (m, 10H), 1.35–1.22 (m, 4H), 1.20–1.09 (m, 2H), 1.23 (s, 6H), 0.91 (s, 6H), 0.88 (s, 6H), 0.82 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  178.8 (2C), 135.2 (2C), 126.2 (2C), 115.0 (2C), 90.6 (2C), 62.9 (2C), 62.9 (2C), 56.5 (2C), 42.6 (2C), 42.3 (2C), 42.0 (2C), 40.7 (2C), 33.2 (2C), 33.0 (2C), 32.8 (2C), 28.4 (2C), 27.0 (2C), 24.2 (2C), 22.4 (2C), 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ESI (*m/z*): calcd for C<sub>44</sub>H<sub>72</sub>N<sub>2</sub>O<sub>6</sub>Na [M + Na]<sup>+</sup>: 747.5294, found 747.5262.

**4.1.34. Preparation of 2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl(1-(2-((1'R,5S,8a'S)-2',5,5',5',8a'-pentamethyl-4,4a',5,5',6',7',8',8a'-octahydro-3H,4'H-spiro[furan-2,1'-naphthalene]-5-yl)ethyl)-1H-1,2,3-triazol-4-yl)methylcarbamate (**37**)**

This compound was prepared by General procedure for Huisgen reaction using 81.0 mg of azide **23** (0.24 mmol) and 43.0 mg of

propynylcarbamate **35** (0.11 mmol) to obtain 51 mg of **37** (64% yield) as a white solid, mp 69–71 °C.  $[\alpha]_D^{20} = -137.1$  (c 1.022, CHCl<sub>3</sub>); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.58 (s, 1H), 5.49 (bs, 2H), 5.27 (bs, 1H), 4.46 (m, 4H), 4.16 (m, 2H), 2.19 (m, 2H), 2.12–1.86 (m, 7H), 1.85–1.70 (m, 8H), 1.75 (s, 3H), 1.73 (s, 3H), 1.71–1.45 (m, 6H), 1.42–1.32 (m, 4H), 1.28–1.20 (m, 3H), 1.27 (s, 3H), 1.21 (s, 3H), 0.91 (s, 3H), 0.90 (s, 6H), 0.89 (s, 3H), 0.81 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 176.2, 156.6, 135.2, 134.7, 126.7 (2C), 126.2 (2C), 122.1, 90.7, 90.2, 81.8, 81.2, 62.9, 47.3, 43.6, 42.6 (2C), 42.3, 42.1 (2C), 40.7 (2C), 39.9, 39.2, 36.3, 33.2, 33.0, 32.9, 32.8, 32.7, 28.4, 28.0, 27.0, 26.9, 24.3, 24.2, 22.4, 21.3 (2C), 18.8 (2C), 16.7 (2C); HRMS-ESI (*m/z*): calcd for C<sub>44</sub>H<sub>70</sub>N<sub>4</sub>O<sub>4</sub>Na [M + Na]<sup>+</sup>: 741.5294, found 741.5292.

#### 4.2. Cells, culture and plating

The human solid tumor cell lines HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast) and WiDr (colon) were used in this study. Cells were maintained in 25 cm<sup>2</sup> culture flasks in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO<sub>2</sub>, 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (100 units penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions displaying >97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 μL per well at densities of 20,000 (WiDr), 15,000 (T-47D) and 10,000 (HeLa, SW1573 and HBL-100) cells per well, based on their doubling times.

#### 4.3. Antiproliferative tests

Chemosensitivity tests were performed using the SRB assay of the NCI with slight modifications. Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicates at different dilutions in the range 1–100 μM. The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which time cells were precipitated with 25 μL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 492 nm, using BioTek's PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium. The percentage growth (PG) was calculated with respect to untreated control cells (C) at each of the drug concentration levels based on the difference in OD at the start (T<sub>0</sub>) and end of drug exposure (T), according to NCI formulas. Therefore, if T is greater than or equal to T<sub>0</sub> the calculation is  $100 \times [(T - T_0)/(C - T_0)]$ . If T is less than T<sub>0</sub> denoting cell killing the calculation is  $100 \times [(T - T_0)/(T_0)]$ . The effect is defined as percentage of growth, where 50% growth inhibition (GI<sub>50</sub>) represents the concentration at which PG is +50. With these calculations a PG value of 0 corresponds to the amount of cells present at the start of drug exposure, while negative PG values denote net cell kill.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2013.06.013>.

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