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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6411–6416
Bioorganic & Medicinal Chemistry Letters

Cytotoxic and apoptotic activities of novel amino analogues of boswellic acids

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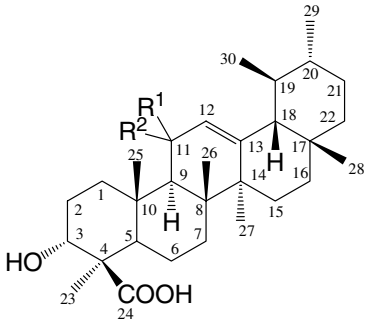
Received 8 March 2007; revised 10 September 2007; accepted 4 October 2007
Available online 10 October 2007

Abstract—4-Amino analogues prepared from β-boswellic acid and 11-keto-β-boswellic acid, wherein the carboxyl group in ursane nucleus was replaced by an amino function via Curtius reaction, displayed improved cytotoxicity than the parent molecules. The same molecules also exhibited apoptotic activity by inducing DNA fragmentation.

The gum exudate of *Boswellia serrata*, used in the treatment of various inflammatory diseases in Ayurvedic system of medicines,[1–3] comprises β-boswellic acid (**1**) as the main triterpenic acid along with 11-keto-β-boswellic acid (**2**) and their acetates.[4] Boswellic acids are known for their therapeutical attributes, as they are reported to be non-redox, non-competitive inhibitors of 5-lipoxygenase.[5–7] The alcoholic extract of the gum is used for the treatment of adjuvant arthritis.[8] It has also shown synergistic effect with glucosamine as anti-inflammatory and anti-arthritic agent.[9] Besides, its activity against ulcerative colitis,[10] chronic colitis,[11] asthma,[12] and anti-complimentary is well documented.[13] The extract has also undergone clinical trials for the treatment of endotoxin induced hepatitis.[14] Recently, β-boswellic acid as a chemo-preventive and therapeutic agent in cancers has also attracted the attention.[15] Semi-synthetic analogues of boswellic acids have also shown promising anticancer activity.[16] Thus, boswellic acids have been reported to inhibit growth of brain tumors[17–19] and induce apoptosis in leukemic cells.[20–22] Using pure topoisomerase assay it was found that both β-boswellic acid acetate and 3-*O*-acetyl-11-keto-β-boswellic acid were more potent inhibitors of topoisomerase I and IIα in comparison to camptothecin and amsacrine or etopside, respectively.[23] The apoptotic effects of boswellic acids have also been observed in malignant glioma cells and colon cancer cells.[17,24]



1. $R^1 = R^2 = H$
2. $R^1 + R^2 = O$

The β-boswellic acid, which belongs to ursane group of triterpenic acids, is lipophilic in nature and comprises only one α-hydroxyl and a carboxyl function. Therefore, to modulate their weakly acidic character and solubility, it was envisaged to modify their structure through the replacement of carboxyl by an amino function, making it weakly basic.

Molecules comprising both amino and alcoholic groups possessing properties of amines as well as alcohols are not only biologically active but also have useful

Keywords: β-Boswellic acid; 11-Keto-β-boswellic acid; Amino analogues; Epimers; Cytotoxicity; DNA fragmentation.
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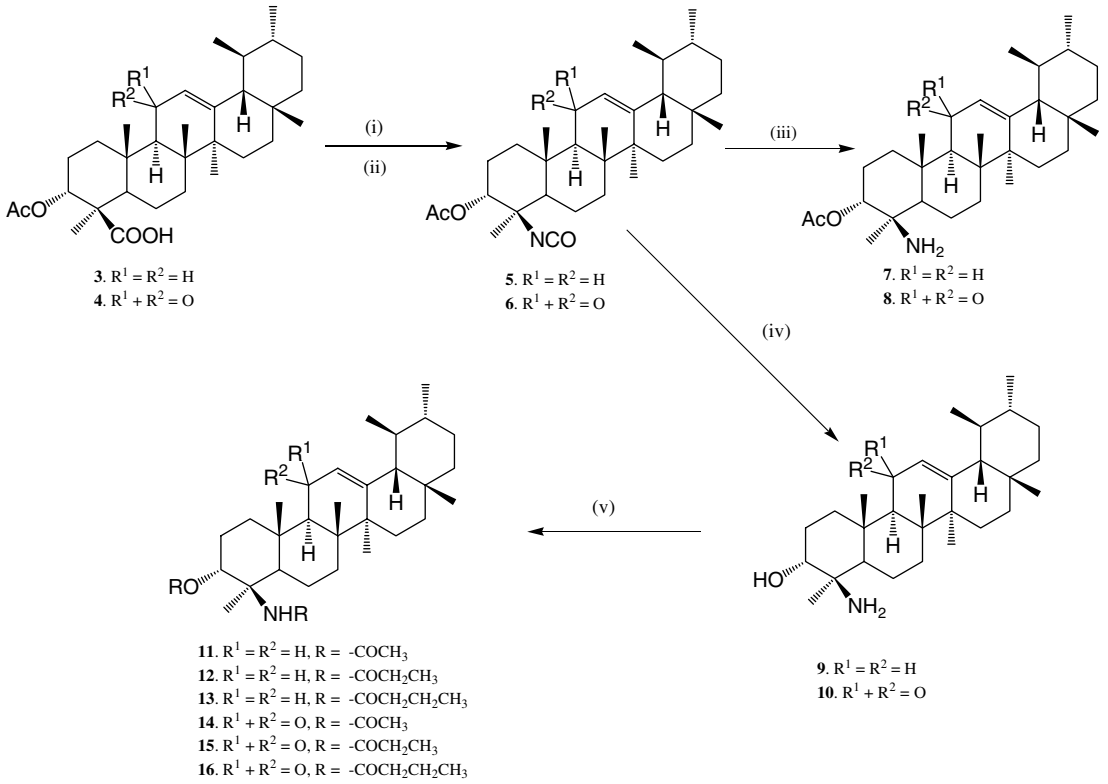
doi:10.1016/j.bmcl.2007.10.011

chemical properties. 1,2-Amino alcohol motif itself is abundant in nature and the most common naturally occurring compounds are vicinal amino alcohols that are biologically active as well as significant members of the chiral pool.[25–27] 1,2-Amino alcohol motif is also a key pharmacophore in aspartyl protease inhibitors, for example, HIV protease inhibitor,[28a] in β-amyloid peptide formation inhibitor for the treatment of Alzheimer's disease,[28b] in dopamine D4 antagonist for the treatment of Parkinson's disease,[28c] and in aldose reductase inhibitor, useful for the management of obesity and diabetes.[28d] Some other well-known examples include bestatin, an aminopeptidase inhibitor that exhibits immunomodulatory activity[29,30] and is used clinically as an adjuvant in cancer chemotherapy.[31] Hapalosin, an anti-β-hydroxy-γ-amino acid containing depsipeptide recently isolated from *bluegreen algae*,[32] has attracted considerable interest due to its ability to inhibit multidrug resistance (MDR) in drug resistant cancer cells. The pyrrolidine amino alcohol anisomycin, obtained from extracts of a *Streptomyces* sp., is a potent inhibitor of protein biosynthesis that may be useful as an anticancer agent.[33–36] Moreover vicinal amino alcohols are also being used as anti-diabetic drugs,[37] in the treatment of heart failures,[38] and as chiral auxiliaries for asymmetric synthesis.[39]

It was therefore envisaged that a small library of novel analogues of boswellic acids (**1**, **2**) thus prepared will be subjected to in vitro cytotoxicity screening against various human cancer cell lines to identify new anticancer lead molecules. In this communication, we describe the synthesis of 3-α-hydroxy-4-β-amino-24-norurs-12-ene derivatives, their epimers from β-boswellic acid, and report their cytotoxic and apoptotic behavior in comparison to natural acids.

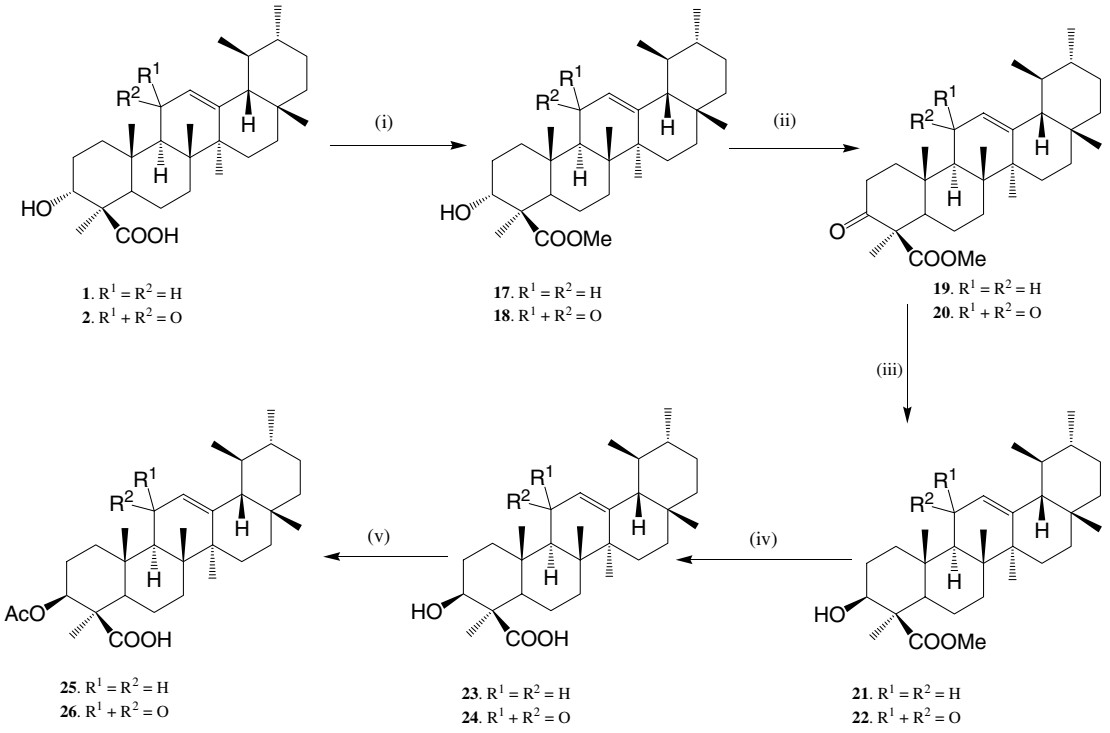
Our synthetic efforts were mainly directed toward the substitution at C-4 with an amino group.[40] Therefore, acetyl β-boswellic **3**/acetyl 11-keto-β-boswellic **4** were subjected to Curtius reaction that initially involved treating **3/4** with $SOCl_2$ followed by reaction with NaN_3 to give isocyanates **5** and **6**. The isocyanate was rearranged to corresponding amine by acid or base treatment. The acid treatment gave 3-α-acetoxy-4-β-amino-24-norurs-12-ene **7** and **8**, but the yields were unsatisfactory. However, the reaction with a base produced 3-α-hydroxy-4-β-amino-24-norurs-12-ene compounds **9** and **10** in good yields, which on acylation in presence of a base gave analogues **11**–**16** (Scheme 1).

Next, epimers of boswellic acids were synthesized following the same reaction sequence as depicted in Scheme 2.[41] First, the acetyl derivatives **3** and **4** were treated with diazomethane (CH_2N_2) to yield methyl esters **17** and **18**, followed by oxidation with PCC to convert to corresponding 3-keto derivatives **19** and **20**. The 3-keto derivatives **19** and **20** were quantitatively reduced by $NaBH_4$ to epimers **21** and **22**, having (S) or β-configuration at C-3, because of the steric hindrance caused by the β-substituent groups mainly in the A/B ring of



3. $R^1 = R^2 = H$
4. $R^1 + R^2 = O$
5. $R^1 = R^2 = H$
6. $R^1 + R^2 = O$
7. $R^1 = R^2 = H$
8. $R^1 + R^2 = O$
9. $R^1 = R^2 = H$
10. $R^1 + R^2 = O$
11. $R^1 = R^2 = H$, R = -$COCH_3$
12. $R^1 = R^2 = H$, R = -$COCH_2CH_3$
13. $R^1 = R^2 = H$, R = -$COCH_2CH_2CH_3$
14. $R^1 + R^2 = O$, R = -$COCH_3$
15. $R^1 + R^2 = O$, R = -$COCH_2CH_3$
16. $R^1 + R^2 = O$, R = -$COCH_2CH_2CH_3$

Scheme 1. Reagents and conditions: (i) $SOCl_2$, benzene, reflux; (ii) NaN_3, acetone, reflux (90%); (iii) HCl/H_2O (1:1) stirring, rt (50%); (iv) KOH, dioxane, reflux (87%); (v) R_2O, DCM, DMAP (95%).

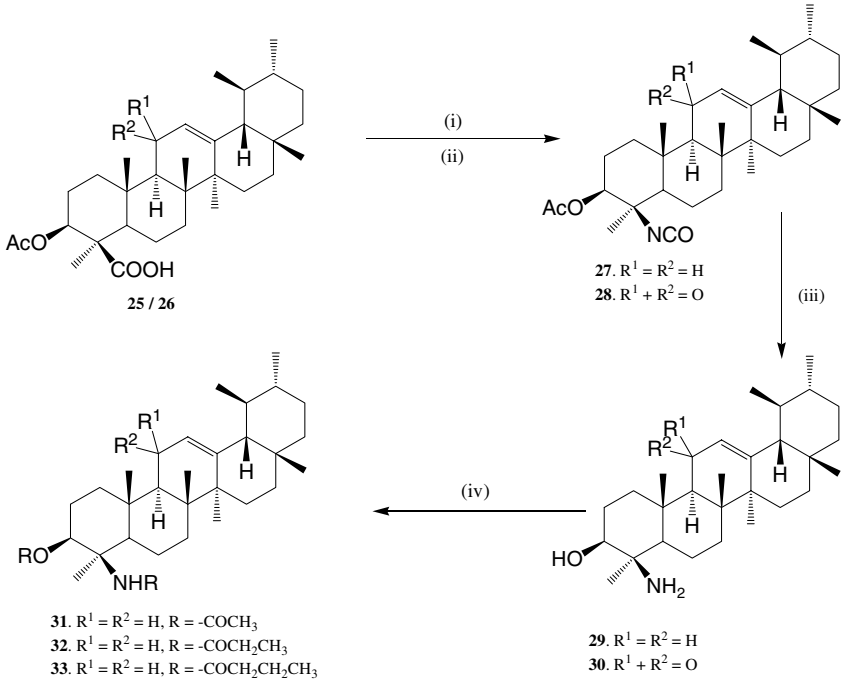


Scheme 2. Reagents and conditions: (i) CH_2N_2, ether (95%); (ii) PCC, DCM, rt (80%); (iii) $NaBH_4$, MeOH, rt (90%); (iv) KOH, MeOH, reflux (88%); (v) Ac_2O, DCM, DMAP (95%).

triterpenoid (e.g., 25-Me, 24-COOMe). The methyl esters thus obtained were hydrolyzed using KOH/MeOH in a high pressure reaction vessel at 98–100 °C to get β-epimeric boswellic acids **23** and **24** in 88% yield, which were then converted to **25** and **26**, respectively, by acetylation reaction.

The β-epimeric boswellic acids **25** and **26** were used as starting material to prepare amino alcohols **27**–**31** as illustrated in Scheme 3.

All the semi-synthetic molecules were subjected to in vitro screening for anticancer activity against vari-



Scheme 3. Reagents and conditions: (i) $SOCl_2$, benzene, reflux; (ii) NaN_3, acetone, reflux (89%); (iii) KOH, dioxane, reflux (86%); (iv) R_2O, DCM, DMAP (95%).

Table 1. In vitro cytotoxicity of natural boswellic acids and novel analogues against human cancer cell lines

Compound	Concn	% Growth inhibition				
		Breast	Prostate	Colon		
		MCF-7	DU-145	SW-620	502713	HT-29
1	1×10^{-5} M	18	00	02	24	15
2	1×10^{-5} M	00	00	07	00	07
3	1×10^{-5} M	00	00	05	20	36
4	1×10^{-5} M	12	10	02	08	24
5	1×10^{-5} M	30	00	00	00	22
6	1×10^{-5} M	10	00	00	02	00
7	1×10^{-5} M	10	**92**	**80**	**96**	44
8	1×10^{-5} M	01	16	**72**	**55**	21
9	1×10^{-5} M	42	**82**	**73**	**99**	**79**
10	1×10^{-5} M	44	**94**	**87**	**86**	**71**
11	1×10^{-5} M	57	08	00	07	00
12	1×10^{-5} M	35	08	00	09	00
13	1×10^{-5} M	00	00	00	07	00
14	1×10^{-5} M	62	09	00	20	00
15	1×10^{-5} M	04	00	00	24	00
16	1×10^{-5} M	00	00	00	12	00
17	1×10^{-5} M	01	13	00	23	00
18	1×10^{-5} M	00	12	01	19	00
19	1×10^{-5} M	00	12	03	27	12
20	1×10^{-5} M	03	13	02	20	05
21	1×10^{-5} M	00	00	12	09	00
22	1×10^{-5} M	00	00	11	10	03
23	1×10^{-5} M	18	01	15	18	11
24	1×10^{-5} M	16	03	01	27	00
25	1×10^{-5} M	15	00	20	12	17
26	1×10^{-5} M	21	00	00	23	19
27	1×10^{-5} M	50	18	06	03	02
28	1×10^{-5} M	09	00	20	00	05
29	1×10^{-5} M	00	16	63	23	51
30	1×10^{-5} M	17	00	07	00	00
31	1×10^{-5} M	02	06	43	00	27
32	1×10^{-5} M	00	11	13	00	24
33	1×10^{-5} M	00	00	21	14	00
5-Fu	5×10^{-5} M	20	31	41	25	36
Mito-C	1×10^{-5} M	65	44	67	84	63
Paclitaxel	1×10^{-5} M	46	53	41	26	76
Adriamycin	1×10^{-6} M	12	46	36	05	14

ous human cancer cell lines as described by Monks et al.[42] In brief, the stock solution (1×10^{-2} M) of the compounds was prepared in dimethylsulfoxide (DMSO) and was further diluted with growth medium (RPMI-1640 with 2 mM glutamine, pH 7.4, 10% fetal calf serum, 100 μg/ml streptomycin, and 100 U/ml penicillin) to obtain desired concentration. The cells were grown in tissue culture flasks in growth medium at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97% viability (Trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 μl of cells (10^5 cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Test materials (100 μl) were then added to the wells and cells were further allowed to grow for another 48 h. The cell growth was stopped by gently layering 50 μl of 50% trichloroacetic acid. The plates were incubated at 4 °C for an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were gently pipetted out and discarded. The plates were washed five times with distilled water and were air-dried. Sulforhodamine B 100 μl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the wells five times with 1% acetic acid. Plates were air-dried, tris-buffer (100 μl, 0.01 M, pH 10.4) was added to all the wells, and plates were gently stirred for 5 min on a mechanical stirrer. The optical density was recorded on ELISA reader at 540 nm. Suitable blanks and positive controls were also included. Each test was done in triplicate. The values reported herein are mean values of three experiments (Table 1). The cytotoxicity of all the compounds was also determined using normal monkey kidney cell line (CV-1) and no significant cytotoxicity was observed (data not included).

The initial results showed that in general high degree of cytotoxic effect was observed at 10 μM with ana-

Table 2. In vitro cytotoxicity of novel analogues **7**, **9**, and **10** at 1 μM concentration

Compound	Concn	% Growth inhibition				
		Breast	Prostate	Colon		
		MCF-7	DU-145	SW-620	502713	Colo-205
7	1×10^{-6} M	0	0	18	6	34
9	1×10^{-6} M	0	3	45	35	30
10	1×10^{-6} M	0	16	**75**	48	48
Mito-C	1×10^{-5} M	78	56	—	—	86
Adriamycin	1×10^{-6} M	74	56	—	—	62

logues **7**–**10** except for breast (MCF-7) cell line. Analogues **9** and **10** showed maximum cytotoxicity (71–99%) against rest of the four cell lines namely prostate (DU-145) and colon (SW-620, 502713, and HT-29). Analogue **7** was equipotent (80–96%) to analogues **8** and **9** for three cell lines namely prostate (DU-145) and colon (SW-620 and 502713) except for colon (HT-29) cell line where it displayed less activity. Compound **8** showed least effect among all the four (analogues **7**–**10**) where maximum effect (72%) was observed with colon (SW-620) cell line. The natural products **1**–**4** have displayed significant cytotoxicity only at 50 μM concentrations (data not included). The amino analogue formation thus resulted in marked improvements in cytotoxicity. It was also established that β-hydroxy epimers and their acylates **21**–**26** and **29**–**33** did not show any improvement in cytotoxicity.

Out of the four analogues **7**–**10**, three analogues **7**, **9**, and **10** were further taken up for cytotoxicity at 1 μM concentration. The results are shown in Table 2. The results clearly indicate that of the three **10** is the most promising which displayed significant activity at 1 μM. The selected analogues **7**–**10** were also analyzed for their apoptotic behavior.

Dysregulation of apoptosis is the hallmark of all cancer cells, and the compounds that are capable of inducing apoptosis in cancer cells are considered to be important in anticancer therapeutics. Apoptosis is a complex phenomenon, which is regulated by genetic mechanisms and is principally characterized by morphological and biochemical changes in the nucleus, including internucleosomal DNA fragmentation. The most active compounds **7**–**10** were analyzed for their potential to induce DNA fragmentation in human promyelocytic leukemia HL-60 cells; the culture was treated with or without 1 μM of test compounds for 6 h. Camptothecin at 1 μM was used as positive control. DNA was extracted from cells and electrophoresed on 1.8% agarose gel. The indicated compounds produced DNA fragments observed in the form of typical ladder of about 180 bp apart, suggesting that all the indicated compounds are capable of producing apoptosis in HL-60 cells (see Fig. 1). Thus the selected molecules are potential leads for the development of anticancer therapeutics.

In conclusion, a series of 29 semi-synthetic 3-amino-4-hydroxy analogues prepared from β-boswellic acid and 11-keto-β-boswellic were subjected to cytotoxicity screening and following interesting observations have been made: (i) 4-α-amino analogues displayed enhanced cytotoxicity than the corresponding carboxylic acid analogues; (ii) protection of free amino group with acyl group reduced the bioactivity; (iii) 3-β-epimeric amino analogues displayed lower activity; (iv) the selected analogues also displayed apoptotic activity as indicated via inducing DNA fragmentation. Further investigations for synthesis and detailed biological activities including mechanism of action are in progress.

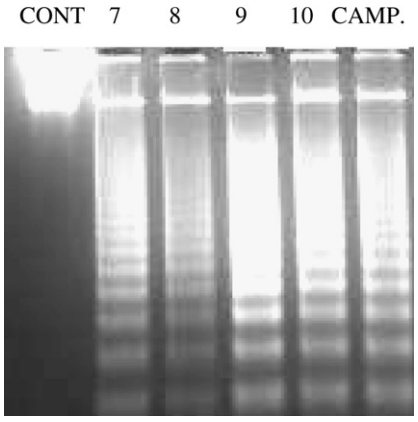


Figure 1. HL-60 cells (2×10^6/3 ml) were treated with analogues of boswellic acids (**7**–**10**) at 1 μM concentration for 6 h. Camptothecin at same concentration and time period was used as positive control. Untreated control cells received the same volume of DMSO as that of treated samples. DNA was extracted and electrophoresed on 1.8% agarose gel.

Acknowledgments

The authors are thankful to CSIR, New Delhi, for supporting the project.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.011.

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40. All compounds provided acceptable data and were identified through ^{1}H NMR and ^{13}C NMR spectroscopy. Compound **5** (3-α-acetoxy-4-β-isocyanato-24-norurs-12-ene): white powder, $[\alpha]_D$ +8° (*c* 0.1 $CHCl_3$). Mp 145–147 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.82, 0.93, 1.08, 1.12, 1.45 (21H, 7× $-CH_3$), 2.11 (3H, s, $-CH_3CO$), 4.78 (1H, br s, H-3), 5.16 (1H, br s, H-12). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 17.1, 17.5, 17.9, 21.3, 21.4, 22.5, 23.1, 23.5, 26.6, 28.1, 28.8, 29.5, 31.3, 32.2, 33.3, 34.6, 37.3, 39.4, 39.7, 39.8, 41.5, 42.3, 47.0, 49.9, 59.1, 60.4, 75.6, 124.4, 127.9, 139.6, 170.8. Anal. Calcd for $C_{32}H_{49}NO_3$: C, 77.53; H, 9.96; N, 2.83. Found: C, 77.43; H, 9.99; N, 2.85. ESI-MS (*m*/*z*): 518 $[M+Na]^+$. Compound **9** (3-α-hydroxy-4-β-amino-24-norurs-12-ene): white powder, $[\alpha]_D$ +7.3° (*c* 0.1 $CHCl_3$). Mp 105–107 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80, 0.91, 1.03, 1.09, 1.19 (21H, 7× $-CH_3$), 3.41 (1H, br s, H-3), 5.14 (1H, t, J = 3.6 Hz, H-12). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.5, 17.0, 17.5, 17.6, 21.5, 23.1, 23.4, 24.9, 26.6, 28.1, 28.7, 28.8, 31.3, 32.6, 33.1, 33.8, 36.8, 39.6, 39.7, 40.2, 41.2, 42.3, 47.4, 48.4, 54.5, 59.1, 76.7, 124.4, 139.6. Anal. Calcd for $C_{29}H_{49}NO$: C, 81.44; H, 11.55; N, 3.27. Found: C, 81.34; H, 11.61; N, 3.22. ESI-MS (*m*/*z*): 428 $[M+H]^+$. Compound **29** (3-β-hydroxy-4-β-amino-24-norurs-12-ene): white powder, $[\alpha]_D$ +6.4° (*c* 0.1 $CHCl_3$). Mp 211–213 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80, 0.91, 0.99, 1.05, 1.09, 1.14 (21H, 7× $-CH_3$), 3.16 (2× d, J = 5.10 Hz, 1H, H-3), 5.14 (br s, 1H, H-12). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.5, 17.5, 18.3, 20.4, 20.9, 22.6, 23.2, 25.5, 26.6, 27.0, 27.8, 28.8, 30.2, 30.7, 31.2, 32.7, 36.5, 39.6, 40.4, 40.7, 41.2, 45.5, 47.1, 50.1, 54.7, 58.5, 79.8, 123.4, 139.7. Anal. Calcd for $C_{29}H_{49}NO$: C, 81.44; H, 11.55; N, 3.27. Found: C, 81.32; H, 11.65; N, 3.23. ESI-MS (*m*/*z*): 428 $[M+H]^+$.
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