Tetrahedron Letters 54 (2013) 4221–4224

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of a novel series of highly functionalized Baylis–Hillman adducts of artemisinin with potent anticancer activity

Abhishek Goswami [a], Partha P. Saikia [a], Bishwajit Saikia [a], Nabin C. Barua [a,*], Ajit K. Saxena [b], Nitasha Suri [b], Madhunika Sharma [b], Gakul Baishya [a]

[a] Natural Products Chemistry Division, CSIR-North-East Institute of Science & Technology, Jorhat 785006, Assam, India
[b] Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Jammu, India

ARTICLE INFO

Article history:
Received 14 March 2013
Revised 24 May 2013
Accepted 28 May 2013
Available online 6 June 2013

Keywords:
Artemisinin
Baylis–Hillman reaction
Cytotoxicity
Deoxoartemisinin
Artemisinin analogs

ABSTRACT

A novel series of densely functionalized derivatives of artemisinin have been synthesized using Baylis–Hillman reaction and their further applicability has been demonstrated. The in vitro anticancer activity of these adducts against a panel of human cancer cell lines is summarized. Compound **10** (% GI of 100 against colon colo-205; % GI of 85 against Lung A-549), **7b** (% GI of 78 against prostrate PC-3) and **9a**, **9b** (% GI of 71 against prostrate PC-3) were especially potent in inhibiting the growth of certain human cancer cell lines and were comparable to that of clinically used anticancer drugs. These newly synthesized compounds can be further utilized as potential precursor for the synthesis of libraries of new artemisinin analogs including dimer.

Artemisinin **1**, a sesquiterpene lactone, isolated from the Chinese medicinal plant *Artemisia annua*[1] and its derivatives, for example, artemether **2** and arteether **3**, are well known antimalarial agents especially against multidrug resistant malarial strains (Fig. 1). The 1,2,4-trioxane system is primarily responsible for its antimalarial activity.[2] In recent years, anticancer activity of artemisinin derivatives has gained attention as a new class of antimalarial and anticancer agents and several promising leads have also emerged from these analogs which are in different stages of development.[3–8]

Because of its sensitive nature, the artemisinin molecule offers very little scope for widespread derivatization. The sensitive nature of the pharmacophore, that is, 1,2,4-trioxane system also demands milder reaction conditions for synthetic manipulation. In this context, several authors have attempted functionalization in the deactivated centers of artemisinin using different microorganisms with limited success, but opened up further prospect of synthetic manipulation. The first generation derivatives of artemisinin viz. C-10 ether or ester derivatives having acetal linkage at C-10 position have been found to be metabolically unstable in the acidic environment of the stomach due to acetal linkage and offer limitation to this class of derivatives. As a result, more and more attention has been diverted to developing C-10 carba analogs to overcome this problem.

Baylis–Hillman reaction is one of the most important carbon–carbon bonds forming reaction with enormous synthetic utility, promise, and potential.[9] The Baylis–Hillman adducts contain at least three functional groups in close proximity which can easily undergo various transformations through appropriate tuning of these groups. The adducts can be tailored in different ways to generate an array of pharmacologically important compounds such as γ-lactam, γ-butyrolactone, epoxides, azides, etc.[10–13] More importantly, the Michael acceptor[14] group present in these adducts offers further scope for addition of various nucleophiles especially artemisinin derived nucleophiles to synthesize artemisinin dimers.

Having known this background and encouraged by the various reports of enhanced biological activity of 10-deoxo artemisinins, we were tempted to synthesize some functionalized artemisinin derivatives utilizing the 10-deoxo artemisinin aldehyde as a

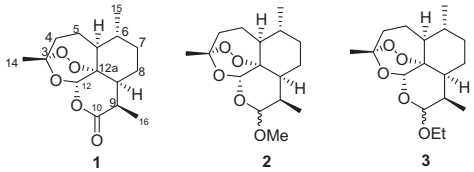
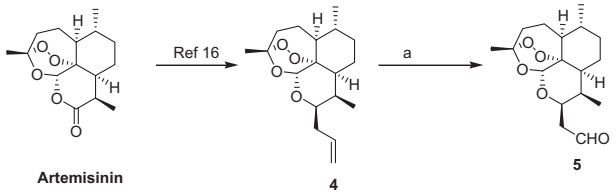


Figure 1.

* Corresponding author. Tel.: +91 376 2371283; fax: +91 376 2370011.
E-mail addresses: ncbarua2000@yahoo.co.in, ncbarua12@rediffmail.com (N.C. Barua).

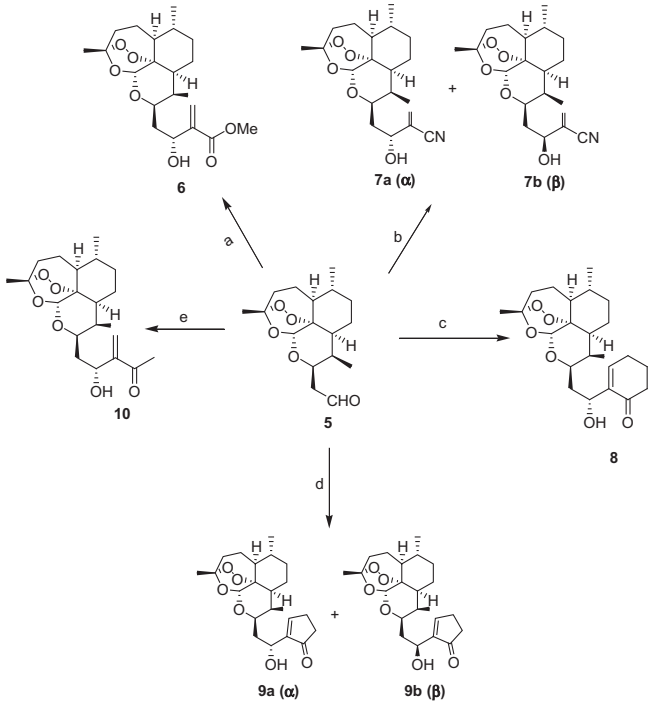
0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.05.123



Scheme 1. Reagents and conditions: (a) osmium tetroxide, 2,6-lutidine, sodium periodate, dioxane–water (1:1), rt, 2 h, 60%.

precursor. As part of our ongoing project on value addition to phytochemicals,[15] we report herein the synthesis of a new series of 10-deoxo artemisinin derivatives along with their preliminary in vitro anticancer activity. It is worth mentioning that Baylis–Hillman reaction on sensitive artemisinin has not been attempted till date.

We designed a series of Baylis–Hillman reaction between 10-deoxo artemisinin aldehyde and different activated olefins in presence of a suitable base to synthesize highly funtionalized artemisinin derivatives. We employed methyl acrylate, acrylonitrile, cyclohexenone, cyclopentenone, and methyl vinyl ketone as the activated olefins in this series of reactions to give rise to products **6**, **7a**, **7b**, **8**, **9a**, **9b**, and **10**, respectively.[16]



Scheme 2. Reagents and conditions: (a) methyl acrylate, DABCO, 31%; (b) acrylonitrile, DABCO, 67%; (c) cylohexenone, DMAP, THF–water, 51%; (d) cyclopentenone, DMAP, THF–water, 60%; (e) methyl vinyl ketone, imidazole, L-proline, THF–water, 60%.

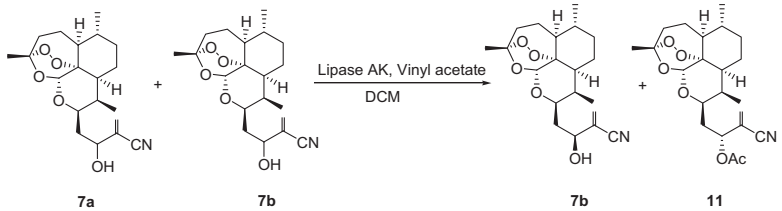
Our synthesis commenced with the three-step conversion of artemisinin to 10-β-allyldeoxo artemisinin **4** following the procedure reported by O'Neill.[17] It was then converted to the 10-deoxo artemisinin aldehyde **5** (Scheme 1) employing the one pot route of dihydroxylation and subsequent cleavage of the diol using osmium tetroxide in presence of 2,6-lutidine and sodium periodate as per procedure reported by Jin and co-workers in 60% yield.[18]

The aldehyde **5** was treated with a variety of activated olefins in presence of different bases (Scheme 2) to get hitherto unknown artemisinin derivatives **6**, **7a**, **7b**, **8**, **9a**, **9b**, and **10**. Treatment of the aldehyde with methyl acrylate in presence of 1, 4-diazabicyclo[2.2.2]octane (DABCO) in solvent free condition generated the adduct **6**. Under similar circumstances acrylonitrile furnished adduct **7**. In case of cyclohexenone and cyclopentenone, Kim and co-workers employed 4-dimethylaminopyridine (DMAP) as the base in catalytic amount.[19] We also tried these conditions with substrate **5** and after 3 days of continuous stirring we could isolate products **8** and **9** in 50–60% yield. Treatment of methyl vinyl ketone with **5** in presence of imidazole and L-proline furnished another densely functionalized artemisinin analog **10** in 60% yield.[20]

Two diastereomers were isolated in case of **7** (dr = 1.5:1; **7a**:**7b**) and **9** (dr = 2:1; **9a**:**9b**)[21] unlike the other cases where the other possible diastereomer was formed in very negligible amount and we did not isolate them. The major less polar isomer was arbitrarily assigned as α-isomer and the minor more polar compound as the β-isomer.[22] Initially, we tried to establish the stereochemistry by NOESY experiments. However, because of the flexibility of the side chain, although we observed indicative NOE cross peaks, it was not considered conclusive. Therefore, we decided to determine the stereochemistry by synthesizing the corresponding MTPA ester. To our surprise, attempts to synthesize MTPA-esters of these compounds were unsuccessful and hence empirical method of Lipase catalyzed kinetic resolution of alcohol put forward by Kazlauskas et al.[23] was adopted. To assign the absolute configuration to the carbinol carbon, an equimolar mixture of **7a** and **7b** was treated with *Pseudomonas fluorescens* Lipase from Amino (Lipase AK) in presence of vinyl acetate (Scheme 3).[24] The major less polar isomer **7a** was seen consumed to give acetylated product **11**. Since the α-isomer has (*R*)-configuration at carbinol carbon, this was in consonance with empirical rule predicted by Kazlauskas.

All the synthesized Baylis–Hillman adducts were tested for in vitro anticancer activity against different cell lines using Sulforhodamine B Assay and the results are summarized in Table 1. Some of the compounds showed encouraging results. Compound **10** showed 100% growth inhibition against colon colo-205 human cancer cell lines. Similarly, the same compound is also found to be promising against lung cancer cells of A-549 type, prostrate PC-3, and neuroblastoma IMR-32 to an extent of 85, 81, and 79 (% GI) respectively. Among other compounds, **7b** showed glimpses of good activity against prostrate PC-3 (% GI 78) and lung A-549 (% GI 77). Appreciable anticancer activities were also shown by **9b** against lung A-549 (% GI 70) and prostrate PC-3 (% GI 71).

The Michael acceptor groups present in these adducts make them susceptible to be attacked by nucleophiles such as nitro

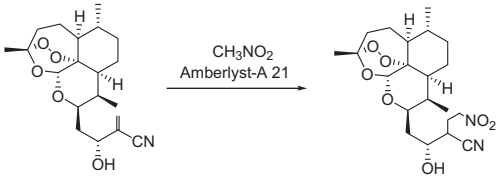


Scheme 3.

Table 1
Proliferation inhibition assay against different cancer cell lines

Compounds	Lung		Ovary	Prostrate		Neuroblastoma	Breast	Colon
	Concd (M)	A-549	IGR-OV-1	DU-145	PC-3	IMR-32	MCF-7	Colo-205
% Growth inhibition								
6	5×10^{-5}	70	63	–	–	56	–	–
7a (α)	5×10^{-5}	66	42	46	65	48	46	56
7b (β)	5×10^{-5}	77	56	60	78	62	55	75
8	5×10^{-5}	66	60	52	68	72	43	63
9a (α)	5×10^{-5}	68	54	47	71	66	51	63
9b (β)	5×10^{-5}	70	52	41	71	57	40	50
10	5×10^{-5}	85	33	73	81	79	66	100
Fluorouracil	2×10^{-5}	–	–	–	–	–	–	54
Paclitaxel	1×10^{-5}	69	–	–	–	–	–	–
Mitomycin	1×10^{-5}	–	–	92	54	–	–	–
Adriamycin	1×10^{-6}	–	–	–	–	70	72	–
Artemisinin	5×10^{-5}	29	26	38	22	23	45	40

'–' Indicates that these compounds were not tested against those cell lines.



Scheme 4.

stabilized carbanion, thiol, amine etc. To validate the probable synthetic routes, Michael addition of nitromethane to **7a** was carried out in presence of Amberlyst A-21 to generate **13** in 80% yield (Scheme 4).

In conclusion, we have synthesized[25] a novel series of artemisinin derivatives using Baylis–Hillman reaction on 10-deoxo artemisinin aldehyde and demonstrated its further applications by 1,4-addition of nitromethane to the Michael acceptor substructure. Further work in this direction is in progress in our laboratory and will be reported in due course of time.

Acknowledgments

We would like to thank Director, NEIST, Jorhat for providing necessary facilities to carry out this work. A.G. thanks UGC and B.S. thank CSIR-New Delhi for the award of fellowship. This is an outcome of CSIR network project NWP-0009.

References and notes

1. Klaymann, D. L. *ACS Symp. Ser.* **1993**, *534*, 242.
2. (a) Klayman, D. L. *Science* **1985**, *228*, 1049; (b) China Co-operative Research Group on Qinghaosu and its Derivatives as Antimalarials *J. Tradit. Chin. Med.* **1982**, *2*, 9.
3. Saikia, B.; Saikia, P. P.; Goswami, A.; Barua, N. C.; Saxena, A. K.; Suri, N. *Synthesis* **2011**, *19*, 3173.
4. (a) Li, Y.; Shan, F.; Wu, J. M.; Wu, G. S.; Ding, J.; Xiao, D.; Yang, W. Y.; Atassi, G.; Le'once, S.; Caignard, D. H.; Renard, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 5; (b) Yang, Z. S.; Zhou, W. L.; Sui, Y.; Wang, J. X.; Wu, J. M.; Zhou, Y.; Zhang, Y.; P. He, L.; Han, J. Y.; Tang, W.; Zuo, J. P.; Li, Y. *J. Med. Chem.* **2005**, *48*, 4608.
5. Yang, Z. S.; Wang, J. X.; Zhou, Y.; Zuo, J. P.; Li, Y. *Bioorg. Med. Chem.* **2006**, *14*, 8043.
6. Slade, D.; Galal, A. M.; Gul, W.; Radwan, M. M.; Ahmed, S. A.; Khan, S. I.; Tekwani, B. L.; Jacob, M. R.; Ross, S. A.; ElSohly, M. A. *Bioorg. Med. Chem.* **2009**, *17*, 7949.
7. Jung, M.; Park, N.; Moon, H.-I.; Lee, Y.; Chung, W.-Y.; Park, K.-K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6303.
8. Chaturvedi, D.; Goswami, A.; Saikia, P. P.; Barua, N. C.; Rao, P. G. *Chem. Soc. Rev.* **2010**, *39*, 435.
9. (a) Basavaiah, D.; Rao, A. J.; Satyanrayana, T. *Chem. Rev.* **2003**, *103*, 811; (b) Basavaiah, D.; Reddy, B. S.; Badsara, S. S. *Chem. Rev.* **2010**, *110*, 5447; (c) Basavaiah, D.; Rao, K. V.; Reddy, R. *J. Chem. Soc. Rev.* **2007**, *36*, 1581.
10. Masuyama, Y.; Nimura, Y.; Kurusu, Y. *Tetrahedron Lett.* **1991**, *32*, 225.
11. (a) Bailey, M.; Marko, I. E.; Ollis, W. D. *Tetrahedron Lett.* **1991**, *32*, 2687; (b) Jackson, R. F. W.; Standen, S. P.; Clegg, W.; McCamley, A. *Tetrahedron Lett.* **1992**, *33*, 6197.
12. Patra, A.; Roy, A. K.; Batra, S.; Bhaduri, A. P. *Synlett* **2002**, 1819.
13. Basavaiah, D.; Rao, J. S. *Tetrahedron Lett.* **2004**, *45*, 1621.
14. (a) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.* **1971**, *14*, 114; (b) Yanagita, M.; Inayama, S.; Kawamata, T.; Ohkura, T.; Herz, W. *Tetrahedron Lett.* **1969**, 4170; (c) Kawamata, T.; Inayama, S. *Chem. Pharm. Bull.* **1971**, *19*, 643; (d) Inayama, S.; Harimaya, K.; Shimizu, N.; Hori, H.; Ohkura, T.; Kawamata, T.; Iitaka, Y. *Heterocycles* **1985**, *23*, 377; (e) Hall, I. H.; William, W. L., Jr.; Grippo, A. A.; Lee, K. H.; Holbrook, D. J.; Chaney, S. G. *Anticancer Res.* **1988**, *8*, 33.
15. Goswami, A.; Saikia, P. P.; Barua, N. C.; Bordoloi, M. J.; Yadav, A.; Bora, T. C.; Gogoi, B. K.; Saxena, A. K.; Suri, N.; Sharma, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 359.
16. (a) Baishya, G.; Barua, N. C.; Goswami, A.; Saikia, P. P.; Rao, P. G.; Saxena, A. K.; Suri, N.; Sharma, M. Patent Filed, 0127DEL2010.; (b) Saikia Bishwajit; Barua Nabin C.; Saikia Partha P.; Goswami Abhishek; Rao Paruchuri G.; Saxena Ajit K.; Suri Nitasha Patent filed, 0366DEL2011 for India & PCT/IN2012/000099.
17. Jeyadevan, J.; Bray, P. G.; Chadwick, J.; Mercer, A. E.; Bryne, A.; Ward, S. A.; Park, B. K.; Williams, D. P.; Cosstick, R.; Davies, J.; Higson, A. P.; Irving, E.; Posner, G. H.; O'Neill, P. M. *J. Med. Chem.* **2004**, *47*, 1290.
18. Yu, W.; Mei, Y.; Kang, Y.; Hua, Z.; Jin, Z. *Org. Lett.* **2004**, *6*, 3217.
19. Lee, K. Y.; Gong, J. H.; Kim, J. N. *Bull. Korean Chem. Soc.* **2002**, *23*, 659.
20. Shi, M.; Jiang, J.-K.; Li, C.-Q. *Tetrahedron Lett.* **2002**, *43*, 127.
21. Diastereomeric ratio was determined from NMR spectra.
22. Ma, J.; Katz, E.; Kyle, D. E.; Ziffer, H. *J. Med. Chem.* **2000**, *43*, 4228.
23. (a) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656; (b) Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 6959; (c) Burges, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129; (d) Kim, M.-J.; Cho, H. J. *J. Chem. Soc., Chem. Commun.* **1992**, 1411; (e) Szigeti, M.; Toke, E. R.; Turóczi, M. C.; Nagy, V.; Szakács, G.; Poppe, L. *Arkivoc* **2008**, *III*, 54.
24. Borah, J. C.; Boruwa, J.; Kalita, B.; Hazarika, A. K.; Barua, N. C. *Indian J. Chem.* **1961**, *2005*, 44B.
25. *Selected analytical data for unknown compounds:* Compound **6** (0.120 g, 31%) as gummy liquid. R_f = 0.2 (1:4 EtOAc/hexane); $[\alpha]_D^{20}$ (*c* 1, $CHCl_3$) +69.5; IR ($CHCl_3$) *ν* 1715, 3458, 2950 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) *δ* 0.82 (d, *J* = 7.4 Hz, 3H, 6-Me), 0.95 (d, *J* = 5.3 Hz, 3H, 9-Me), 1.41 (s, 3H, 3-Me), 2.20–2.07 (m, 12H, arte aliphatic), 2.33 (dt, *J* = 3.4, 12.4 Hz, 1H), 2.65 (sextet, 1H), 3.33 (d, *J* = 7.0 Hz, 1H), 3.77 (s, 3H), 4.48 (m, 1H, H-10), 4.77 (br s, 1H), 5.36 (s, 1H, H-12), 6.02 (s, 1H, C=CH_2), 6.33 (s, 1H, C=CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) 12.7, 19.9, 24.3, 24.4, 25.7, 29.6, 33.9, 34.0, 36.2, 37.1, 44.0, 51.5, 51.9, 68.5, 71.6, 80.6, 88.6, 103.0, 125.3, 141.8, 166.4; ESIMS *m*/*z* 396.2 (M^+); [found: C, 63.53; H, 8.21. $C_{21}H_{32}O_7$ calcd C, 63.62; H, 8.14].
Compound ***7a*** *(α):* solid, mp = 137 °C, R_f = 0.4 (1:3 EtOAc/hexane), $[\alpha]_D^{20}$ (*c* 1.1, $CHCl_3$) +13.2, IR ($CHCl_3$) *ν* 3469, 2926 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) *δ* 0.88 (d, *J* = 7.4 Hz, 3H, 6-Me), 0.96 (d, *J* = 4.2 Hz, 3H, 9-Me), 1.40 (s, 3H, 3-Me), 1.15–2.07 (m, 12H, arte aliphatic), 2.32 (dt, *J* = 3.4, 12.4 Hz, 1H), 2.66 (sextet, 1H), 4.38 (br s, 1H, O*H*), 4.45 (m, 1H), 4.66 (m, 1H), 5.37 (s, 1H, H-12), 6.00 (s, 1H, C=CH_2), 6.14 (s, 1H, C=CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) 11.6, 12.4, 20.0, 24.7, 25.9, 30.6, 35.5, 36.4, 37.5, 43.4, 51.7, 73.1, 74.8, 80.9, 89.6, 103.1, 125.7, 129.9, 144.1, 188.2; ESIMS: *m*/*z* 386.3 (M^++Na); [found: C, 66.12; H, 8.07; N, 3.81. $C_{20}H_{29}NO_5$ calcd C, 66.09; H, 8.04; N, 3.85].
Compound ***7b*** *(β):* Gummy, R_f = 0.3 (1:3 EtOAc/hexane) $[\alpha]_D^{20}$ (*c* 1.0, $CHCl_3$) +33.0, IR ($CHCl_3$) *ν* 3468, 2925 cm^{-1}, ^1H NMR ($CDCl_3$, 300 MHz) *δ* 0.87 (d, *J* = 7.4 Hz, 3H, 6-Me), 0.96 (d, *J* = 5.2 Hz, 3H, 9-Me), 1.66 (s, 3H, 3-Me), 1.25–2.17 (m, 12H, arte aliphatic), 2.32 (dt, *J* = 3.4, 12.4 Hz, 1H), 2.66 (sextet, 1H), 3.63 (br s, 1H, O*H*), 4.59 (m, 2H), 5.37 (s, 1H, H-12), 6.09 (s, 1H, C=CH_2), 6.24 (s, 1H, C=CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 12.7, 20.1, 24.7, 25.9, 30.0, 33.3, 34.2, 36.5, 37.5, 43.8, 51.9, 70.0, 70.9, 80.8, 89.3, 103.2, 122.0, 130.5, 133.0, 190.5;

ESIMS: m/z 363.2 (M^+); [found: C, 66.15; H, 8.07; N, 3.79. $C_{20}H_{29}NO_5$ calcd C, 66.09; H, 8.04; N, 3.85].

Compound **8**: Solid, R_f = 0.6 (1:3 EtOAc/hexane) mp = 134 °C, $[\alpha]_D^{20}$ (c 1.1, $CHCl_3$) −3.4; IR ($CHCl_3$) ν 1726, 2924, 3495 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.92 (d, J = 7.3 Hz, 3H, 6-Me), 0.95 (d, J = 6.2 Hz, 3H, 9-Me), 1.39 (s, 3H, 3-Me), 1.23–2.04 (m, 15H, arte aliphatic), 2.39–2.43 (m, 3H), 2.68 (m, 1H, H-9), 3.72 (m, 1H, CH_2–CHOH), 4.13 (q, J = 7.1 Hz, 1H, H-10), 4.27 (br s, 1H, CH_2–CHOH), 4.99 (d, J = 9.8 Hz, 1H, CH-OH), 5.23 (s, 1H, H-12), 7.18 (t, J = 3.8 Hz, 1H, C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.5, 20.2, 21.2, 22.8, 24.8, 25.7, 26.0, 29.5, 34.1, 36.0, 37.4, 38.0, 38.6, 45.8, 51.6, 65.4, 73.9, 80.8, 91.8, 104.4, 141.3, 145.6, 199.3; ESIMS m/z 406.2 (M^+); [found: C, 67.92; H, 8.39. $C_{23}H_{34}O_6$ calcd C, 67.96; H, 8.43].

Compound **9a** (α): Gummy; R_f = 0.5 (1:3 EtOAc/hexane); $[\alpha]_D^{20}$ (c 0.5, $CHCl_3$) −17.1; IR ($CHCl_3$) ν 1726, 2918, 3469 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.89 (d, J = 7.1 Hz, 3H, 6-Me), 0.91 (d, J = 5.8 Hz, 3H, 9-Me), 1.39 (s, 3H, 3-Me), 1.25–2.03 (m, 13H, arte aliphatic), 2.17–2.42 (m, 3H), 2.43–2.60 (m, 2H), 3.75 (m, 1H), 4.27 (m, 1H, CH_2–CHOH), 4.88 (d, J = 9.3 Hz, 1H, CH_2-CHOH), 5.23 (s, 1H, H-12), 7.65 (s, 1H, C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.6, 20.2, 21.2, 24.7, 26.0, 26.5, 29.6, 34.1, 35.5, 36.0, 36.7, 37.4, 45.7, 51.6, 64.5, 73.7, 80.8, 91.8, 104.4, 148.5, 158.5, 208.9; ESIMS: m/z 415.8 (M^++Na); [found: C, 67.28; H, 8.19. $C_{22}H_{32}O_6$ calcd C, 67.32; H, 8.22].

Compound **9b** (β): Gummy, R_f = 0.4 (1:3 EtOAc/hexane); $[\alpha]_D^{20}$ (c 0.7, $CHCl_3$) +22.7; IR ($CHCl_3$) ν 1696, 2918, 3436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.84 (d, J = 7.3 Hz, 3H, 6-Me), 0.91 (d, J = 5.8 Hz, 3H, 9-Me), 1.39 (s, 3H, 3-Me), 1.25–2.16 (m, 13H, arte aliphatic), 2.30 (dt, J = 3.4, 12.4 Hz, 1H), 2.44 (m, 1H), 2.61–2.67 (m, 3H), 3.41 (d, J = 6.1 Hz, 1H, CH_2–CHOH), 4.36–4.41 (m, 1H, H-10), 4.72 (br s, 1H, CH_2–CHOH), 5.37 (s, 1H, H-12), 7.63 (s, 1H, C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.0, 20.2, 21.9, 24.6, 26.0, 26.5, 29.9, 34.2, 35.1, 35.6, 36.5, 37.4, 46.2, 52.3, 66.4, 72.2, 81.0, 95.9, 103.4, 148.0, 159.0, 208.8; ESIMS: m/z 392.2 (M^+); [found: C, 67.31; H, 8.24. $C_{22}H_{32}O_6$ calcd C, 67.32; H, 8.22].

Compound **10**: R_f = 0.45 (1:2 EtOAc/hexane); $[\alpha]_D^{20}$ (c 0.45, $CHCl_3$) +14.4; IR (neat) ν 1717, 2923, 3481 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.74 (d, J = 7.1 Hz, 3H, 6-Me), 0.95 (d, J = 6.0 Hz, 3H, 9-Me), 1.38 (s, 3H, 3-Me), 1.20–2.20 (m, 13H, arte aliphatic), 2.31 (m, 1H), 2.36 (s, 3H, –CH_3), 3.77 (t, J = 6.5 Hz, 1H, CH_2–CHOH), 4.49 (br s, 1H, –OH), 4.78 (d, J = 9.0 Hz, 1H, CH_2–CHOH), 5.29 (s, 1H, H-12), 6.18 (s, 1H, C=CH_2), 6.30 (s, 1H, C=CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.6, 20.2, 21.2, 24.7, 25.9, 28.4, 29.5, 30.0, 34.0, 36.1, 37.3, 45.9, 51.7, 75, 80.5, 91.8, 104.7, 122, 188.2, 204.5; ESIMS: m/z 380.2 (M^+); [found: C, 66.31; H, 8.43. $C_{21}H_{32}O_6$ calcd C, 66.29; H, 8.48].

Compound **11**: as a gum in 40% yield. IR ($CHCl_3$) ν 1731, 3469, 2926 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.91 (d, J = 7.4 Hz, 3H, 6-Me), 0.96 (d, J = 4.2 Hz, 3H, 9-Me), 1.42 (s, 3H, 3-Me), 1.18–2.17 (m, 12H, arte aliphatic), 2.1 (s, 3H, CH_3), 2.32 (dt, J = 3.4, 12.4 Hz, 1H), 2.68 (sextet, 1H), 4.41 (br s, 1H, OH), 4.46 (m, 1H), 4.71 (m, 1H), 5.37 (s, 1H, H-12), 6.04 (s, 1H, C=CH_2), 6.11 (s, 1H, C=CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz) 11.6, 12.4, 20.0, 24.7, 25.9, 30.6, 35.5, 36.4, 37.5, 43.4, 51.7, 73.1, 74.8, 80.9, 89.6, 103.1, 125.7, 129.9, 144.1, 171.2, 188.2; ESIMS: m/z 405.2 (M^+); [found: C, 65.12; H, 7.74; N, 3.51. $C_{22}H_{31}NO_6$ calcd C, 65.17; H, 7.71; N, 3.45].

Compound **13**: (0.093 g, 80%) as gum. R_f = 0.3 (1:2 EtOAc/hexane); $[\alpha]_D^{20}$ (c 2.5, $CHCl_3$) +29.1; IR ($CHCl_3$) ν 3469, 2926, 1556 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.90 (d, J = 7.5 Hz, 3H, 6-Me), 0.98 (d, J = 5.2 Hz, 3H, 9-Me), 1.40 (s, 3H, 3-Me), 1.25–2.03 (m, 14H, arte aliphatic), 2.31 (m, 1H), 2.58 (dt, J = 7.1, 14.0 Hz, 1H), 2.83 (sextet, 1H), 3.98 (m, 1H, H-10), 4.42 (br s, 1H, OH), 4.58–4.70 (m, 3H, CH_2–CHOH+CH_2NO_2), 5.35 (s, 1H, H-12). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.3, 20.2, 24.8, 25.8, 26.0, 32.8, 34.2, 35.9, 36.3, 37.2, 37.4, 43.3, 51.7, 72.8, 74.5, 80.9, 91.7, 104.5, 119.0; ESIMS: m/z 424.2 (M^+); [found: C, 59.47; H, 7.56; N, 6.64. $C_{21}H_{32}N_2O_7$ calcd C, 59.42; H, 7.60; N, 6.60].