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Hemisynthesis and Preliminary Evaluation of Novel Endocannabinoid Analogues

Siham El Fangour,[a] Laurence Balas,[a,*] Jean-Claude Rossi,[a] Andrey Fedenyuk,[b] Natalia Gretskaya,[b] Mikhail Bobrov,[b] Vladimir Bezuglov,[b] Cecilia J. Hillard[c] and Thierry Durand[a]

[a]*UMR CNRS 5074, Faculté de Pharmacie, 15 av. C. Flahault, BP 14491, F-34093 Montpellier Cedex 5, France*
[b]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, 16/10 Miklukho-Maklaya str., 117437 Moscow, Russia*
[c]*Department of Pharmacology and Toxicology, Medicinal College of Wisconsin, Milwaukee, WI 53226, USA*

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Abstract—Three new endocannabinoid analogues in which amide moiety was replaced either by oxomethylene group or ester moiety with simultaneous substitution of both α-hydrogens with methyl groups were synthesized and their abilities to interact with CB1-receptor and FAAH were investigated.

Endocannabinoids[1] are endogenous ligands of cannabinoid receptors which, interestingly are less psychoactive than Δ^9-tetrahydrocannabinol (Δ^9-THC), the well-known active component of marijuana. Thus, since the discovery in 1992 of the first endocannabinoid, named anandamide[2] **1**, research on cannabimimetic drugs took an important revival of interest.

To date, several new families of endocannabinoids were identified in central and peripherical nervous systems: while two of them (2-AG[3] **2** and virodhamine[4]) are arachidonate esters of glycerol and ethanolamine, respectively, an ether linkage confers a higher metabolic stability to noladin ether **3**.[5] In addition, among the amide family containing anandamide **1** as a leader, oleamide (amide of oleic acid) that accumulates in the cerebrospinal fluid under conditions of sleep deprivation and induces physiological sleep in animals,[6] could be referred as a prototypical member of this family. Several aminoacid derivatives: AA-Gly, AA-GABA and AA-Ala with analgesic activity were recently found in bovine and rat brains.[7] Besides, cannabimimetic arachidonoyldopamine,[8] which was recently proposed to be endogenous activator of vanilloid receptor,[9] significantly expands the physiological role of the mentioned amide family.

Acting on cannabinoid (CB1/CB2) receptors, endocannabinoids have been shown to influence the activity of nervous, vascular and immune systems. Thus, interest in endocannabinoids as therapeutic agents[10,11] has led us to design novel anandamide analogues that might be resistant to hydrolases degradation.

Various structure–affinity and structure–activities relationship studies for the CB1 receptor,[12] the anandamide transporter[13,14] and the fatty acid amide hydrolase (FAAH) have been reported.[15,16]

Thus, numerous endocannabinoid analogues were synthesized as tools to probe the influence of the lipid chain, the importance of the carbonyl function and the tolerance to steric hindrance on the polar head moiety. Modifications of the amide linkage were also investigated: esters,[17,14] carbamates,[18] ureas,[18] ethers,[18] methylene-linked, ketone[19] and amine[20] derivatives as either anandamide or 2-AG analogues.

Keeping in mind all the above data, we based our investigations on template of arachidonoyl-ethyleneglycol **4** (AA-EG). Thus, we report herein the synthesis of the novel arachidonyl derivatives **6** and **5** together with its linoleyl derivative **9**.

AA-EG **4**, firstly prepared[21] as 2-AG analogue, possesses true cannabimimetic properties[22] and can be regarded as 'promiscuous' analogue of both ananda-

*Corresponding author. Tel.: +33-4-67-54-86-24; fax: +33-4-67-54-86-25; e-mail: balas@pharma.univ-montp1.fr

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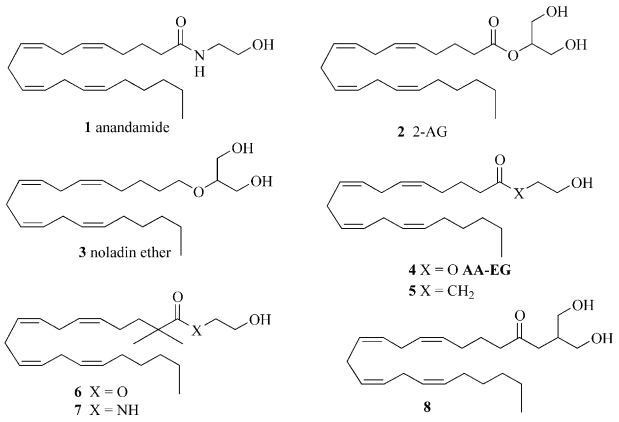
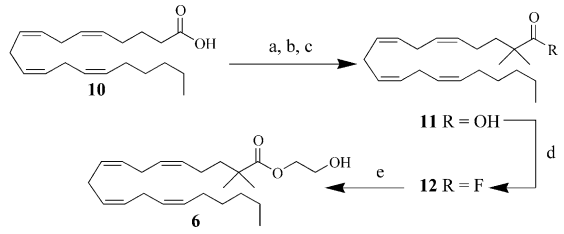
mide **1**, in which amide moiety has been replaced with ester functionality, or 2-AG **2**, simplified by replacement of one hydroxymethylene group with hydrogen.



First, we decided to protect the ester bond of AA-EG **4** by steric hindrance at the α-position to the carbonyl functionality created by two methyl groups as in analogue **6**. Such a strategy was proved to be successfully efficient on anandamide **1** leading to structure **7**.[23]

The synthesis of analogue **6**[24] was afforded, in 62% yield,[25] by coupling α,α-dimethyl arachidonic acid **11** with ethylene glycol via intermediate acyl fluoride **12** according to our published procedure.[22] Acid **11** was synthesized from arachidonic acid **10** by esterification, followed by two runs of known[23] condensation of methyliodide to α-lithium derivative and then saponification (Scheme 1).



Scheme 1. Synthesis of the dimethyl ester analogue **6**: (a) CH_2N_2, MeOH, 0 °C, 20 min, 100%; (b) LDA, THF, −80 °C, 1 h then MeI, −70 to −30 °C, 1.5 h followed by LDA, THF, −80 °C, 1 h then MeI, −70 to −30 °C, 1.5 h, 77%; (c) NaOH, THF, MeOH, 50 °C, 7.5 h, 98%; (d) cyanuric fluoride, Py, MeCN, 23 °C, 1 h; (e) ethylene glycol, DMAP, MeCN, 23 °C, 18 h, 62% from **11**.

Second, we envisioned to prevent the analogues from hydrolase degradation by replacement of the CO–NH bond of anandamide **1** by a CO–CH_2 linkage. Sugiura et al. have already published[19] the total synthesis of keto-analogue **8** of 2-AG. We thought that hemisynthesis would offer a more concise route to ketone analogues **5** than the one presented by Sugiura et al.[19] (10 steps) for preparation of compound **8**.

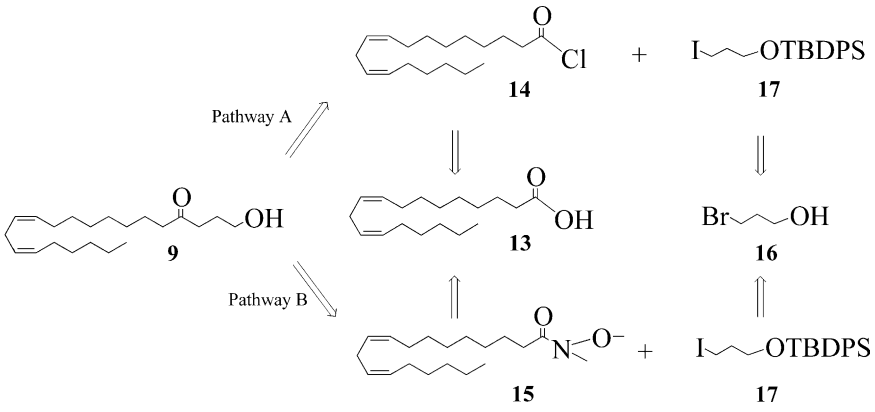
Investigations towards the synthesis of the ketone derivatives were performed using linoleic acid **13** prior to apply our best results to arachidonic acid **10**, much more expensive.

Two approaches were considered for the carbon-carbon bond formation (Scheme 2): Pathway A relies upon organozinc cross-coupling to acid chloride **14** while pathway B is based on carbanion condensation to the Weinreb amide **15**. Thus, both strategies required the same commercially available starting material that is, 3-bromo-1-propanol **16** and the desired free polyunsaturated fatty acid. The choice of the TBDPS protection was guided by its readily UV detection to monitor both organozinc and carbanion intermediates formation.

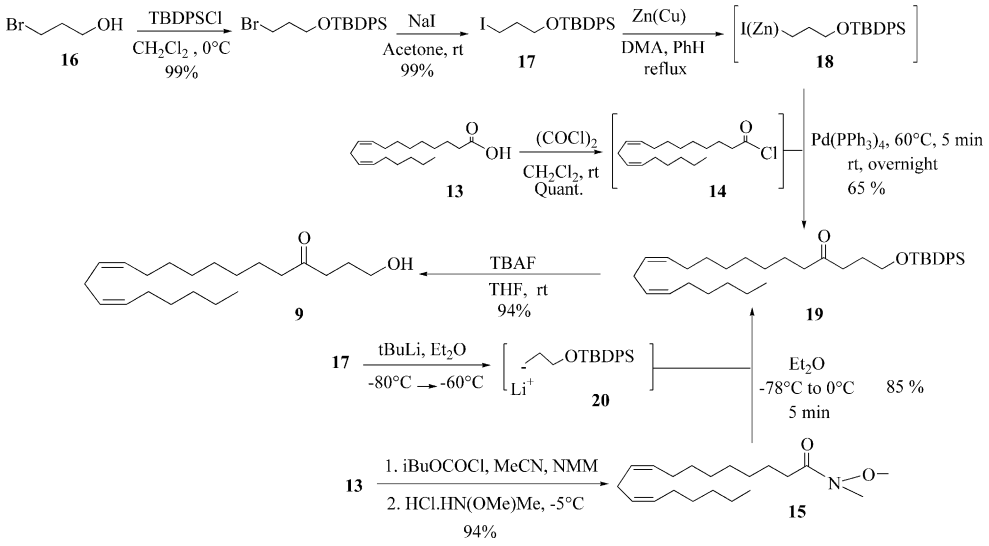
Pathway A is highlighted in Scheme 3. Thus, *O*-silylation of bromopropanol **16**, followed by Finkelstein halogen-exchange reaction produced 3-iodopropyl *tert*-butyldiphenylsilyl ether **17** in 98% yield.

Unfortunately, all our initial efforts to carry out palladium cross-coupling reaction between the crude linoleoyl chloride **14** (0.7 mmol) and organozinc intermediate **18** (according to Tamaru et al. procedure[26]) afforded ketone **19** in less than 30% yield. Surprisingly, such bad yields were also obtained with freshly purified methyl 5-chloro 5-oxovalerate and methyl 4-chloro-4-oxobutyrate as models. It was subsequently found that efficiency in the preparation of this ketone could be nicely improved when carrying out the chemistry on larger scale. Effectively, the synthesis of ketone **19** reached 65% yield using 5.0 mmol of linoleic acid **13**.

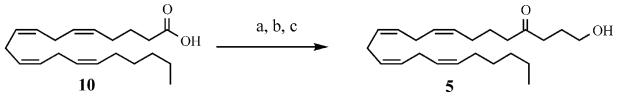
In our hands, such a molar size dependence is therefore financially cumbersome for arachidonic acid.



Scheme 2. Retrosynthetic analysis.



Scheme 3. Synthesis of linoleyl-ketone **9**.



Scheme 4. Synthesis of arachidonyl-ketone **5:** (a) iBuOCOCl, MeCN, NMM, 0 °C, 40 min then HCl. HN(OMe)Me, −15 °C, 82%; (b) generation of carbanion **20**: ^{t}BuLi, **17**, Et_2O, −80 to −60 °C, then condensation: Et_2O, −78–0 °C, 15 min; (c) TBAF, THF, 96%.

Thus, the difficulties encountered with pathway A prompted us to examine pathway B. This later strategy is outlined in Scheme 3.

Linoleic acid **13** was readily converted to the Weinreb amide **15** using the mixed anhydride method.[8] Thus, treatement of the fatty acid with isobutyl chloroformate followed by *N*,*O*-dimethylamine and *N*-methylmorpholine furnished the corresponding amide **15** in excellent yield. Condensation of the lithiated carbanion **20** to the *N*-methoxy-*N*-methylamide **15** provided the expected ketone **19** in 85% yield. The TBDPS protecting group of ketone **19** was removed by treatement with TBAF to produce the expected linoleyl keto-alcohol **9** in 94% yield.[27]

Application of pathway B to arachidonic acid **10** provided target ketone **5**[28] (Scheme 4) in only 3 steps in overall 27% yield (non optimized).

Abilities of novel compounds **5**, **6**, and **9** to interact with CB1-receptor and FAAH were then investigated using rat forebrain membranes according to Jarrahian et al. procedure.[13] The results are summarized in Table 1.

Arachidonyl-ketone **5** exhibits a moderate affinity for the CB1 receptor with a K_i value 4-fold higher than that of anandamide **1**, while linoleyl ketone **9** and both esters **4** and **6** were inactive.

Moreover, compound **6** displays an interesting inhibitory effect on the FAAH activity (very close to anandamide **1**), while the presence of the keto-compounds **5** and **9** does not influence it.

As a conclusion, three novel anandamide analogues **6**, **5**, and **9** were synthesized. The evaluation of their affinity for the CB1 cannabinnoid receptor and FAAH inhibition was presented as preliminary biological results. Ability of these three analogues to bind to the CB2 receptor and the anandamide carrier (ANT) together with their pharmacological behavior in the tetrad test in mice are currently under progress. To argue a SAR study, the synthesis of other keto-analogues, especially virodhamine and AA-Gly analogues, is under investigation too.

Table 1. Evaluation of the cannabinoid properties of analogues **5**, **6** and **9** in comparison with anandamide **1** and arachidonoylethylene glycol **4**

Compd (1 μM)	% CB1 affinity[b] (PMSF)	CB1 affinity (PMSF) K_i[a,b]	FAAH activity[c] at 1 μM	FAAH activity at 10 μM	FAAH activity[a] K_i
1	19	90 nM (±32)			3 μM (±0.5)
4	83	>10 μM[d]	100	70	
5	38	360 (±10 nM)	91	78	
6	100	>10 μM	90	12	2.1 (±0.1 μM)
9	72	2.1 (±1 μM)	100	100	

[a]Values are means of three experiments, standard deviation is given in parentheses.
[b]Competition with [^{3}H]-CP55940 agonist.
[c]Percentage of anandamide hydrolysis in the presence of tested compounds.
[d]PMSF is not an effective inhibitor of mono-acyl glycerol lipase and other lipases, possibly responsible for AA-EG hydrolysis.

Acknowledgements

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References and Notes

1. Mechoulam, R.; Fride, E.; Marzo, V. D. *Eur. J. Pharmacol.* **1998**, *359*, 1.
2. Devane, W. A.; Hanuš, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946.
3. (a) Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almog, S.; Martin, B. R.; Compton, D. R.; Pertwee, R. G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. *Biochem. Pharmacol.* **1995**, *50*, 83. (b) Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89.
4. Porter, A. C.; Sauer, J. M.; Knierman, M. D.; Becker, G. W.; Berna, M. J.; Bao, J.; Nomikos, G. G.; Carter, P.; Bymaster, F. P.; Leese, A. B.; Felder, C. C. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1020.
5. Hanuš, L.; Abus-Lafi, S.; Fride, E.; Breur, A.; Vogel, Z.; Shalev, D. E.; Kustanovich, I.; Mechoulam, R. *PNAS* **2001**, *98*, 3662.
6. Cravatt, B. F.; Prospero-Garcia, O; Siuzdak, G.; Gilula, N. B.; Henriksen, S. J.; Boger, D. L.; Lerner, R. A. *Science* **1995**, *268*, 1506.
7. Huang, S. M.; Bisogno, T.; Petros, T. J.; Chang, S. Y.; Zavitsanos, P. A.; Zipkin, R. E.; Sivakumar, R.; Coop, A.; Maeda, D. Y.; De Petrocellis, L.; Burstein, S.; Di Marzo, V.; Walker, J. M. *J. Biol. Chem.* **2001**, *276*, 42639.
8. Bezuglov, V.; Bobrov, M.; Gretskaya, N.; Gonchar, A.; Zinchenko, G.; Melck, D.; Bisogno, T.; Di Marzo, V.; Kuklev, D.; Rossi, J.-C.; Vidal, J.-P.; Durand, T. *Biomed. Chem. Lett.* **2001**, *11*, 447.
9. Huang, S. M.; Bisogno, T.; Trevisani, M.; Al-Hayani, A.; De Petrocellis, L.; Fezza, F.; Tognetto, M.; Petros, T. J.; Krey, J. F.; Chu, C. J.; Miller, J. D.; Davies, S. N.; Geppetti, P.; Walker, J. M.; Di Marzo, V. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 8400.
10. Mechoulam, R.; Panikashvili, D.; Shohami, E. *Trends in Molecular Medicine* **2002**, *8*, 58.
11. Goutopoulos, A.; Makriyannis, A. *Pharmacol. Ther.* **2002**, *95*, 103.
12. Regio, P. H. *Prostaglandins Leukot. Essent. Fatty Acids* **2002**, *66*, 143.
13. Jarrahian, A.; Manna, S.; Edgemond, W. S.; Cambell, W. B.; Hillard, C. J. *J. Neurochem.* **2000**, *74*, 2597.
14. Lopez-Rodriguez, M. L.; Viso, A.; Ortega-Gutierrez, S.; Lastres-Becker, I.; Gonzalez, S.; Fernandez-Ruiz, J.; Ramos, J. A. *J. Med. Chem.* **2001**, *44*, 4505.
15. Khanolkar, A. D.; Makriyannis, A. *Life Sciences* **1999**, *65*, 607.
16. Van der Stelt, M.; Van Kuik, J. A.; Bari, M.; Van Zadelhoff, G.; Leeflang, B. R.; Veldink, G. A.; Finazzi-Agro, A.; Vliegenthart, J. F.; Maccarrone, M. *J. Med. Chem.* **2002**, *45*, 3709.
17. Sugiura, T.; Kodaka, T.; Nakane, S.; Miyashita, T.; Kondo, S.; Suhara, Y.; Takayama, H.; Waku, K.; Seki, C.; Baba, N.; Ishima, Y. *J. Biol. Chem.* **1999**, *274*, 2794.
18. Ng, E. W.; Aung, M. M.; Abood, M. E.; Martin, B. R.; Razdan, R. K. *J. Med. Chem.* **1999**, *42*, 1975.
19. Suhara, Y.; Nakane, S.; Arai, S.; Takayama, H.; Waku, K.; Ishima, Y.; Sugiura, T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1985.
20. Berglund, B. A.; Boring, D. L.; Wilken, G. H.; Makriyannis, A.; Howlett, A. C.; Lin, S. *Prostaglandins Leukot. Essent. Fatty Acids* **1998**, *59*, 111.
21. Sugiura, T.; Kondo, S.; Sukagava, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1995**, *512*, 89.
22. Bezuglov, V. V.; Bobrov, M.Yu.; Gretskaya, N. M.; Archakov, A. V.; Serkov, I. V.; Fedenuyk, A. P.; Verevochkina, E.Yu.; Kogteva, G. S.; Titova, O.Yu.; Marvanov, D. M.; De Petrocellis, L.; Bisogno, T.; Di Marzo, V.; Manevich, Y. *Russian J. Bioorg. Chem.* **1998**, *24*, 833.
23. Sheskin, T.; Hanuš, L.; Slager, J.; Vogel, Z.; Mechoulam, R. *J. Med. Chem.* **1997**, *40*, 659.
24. Colorless oil; TLC R_f 0.44 (benzene/ethylacetate 4/1); ^{1}H NMR ($CDCl_3$, 600 MHz) δ(ppm): 0.89 (t; 3H, $J=7$ Hz; H_2O); 1.22 (s, 6H, α-CH_3); 1.15–1.4 (m, 6H, H17, H18, H19); 1.55–1.64 (m, 2H, H3); 1.97–2.09 (m, 4H, H4, H16); 2.75–2.87 (m, 6H, H7, H10, H13); 3.82 (br s, 2H, H2′); 4.21 (t, 2H, $J=5$ Hz, H1′); 5.29–5.43 (m, 8H, H5, H6, H8, H9, H11, H12, H14, H15). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 14.1 (C20); 22.6 (C4); 23.0 (C19); 25.1 (2 α-Me); 25.5–25.6–27.2–29.3–31.5 (C3, C7, C10, C13, C16, C17, C18); 40.5 (C2); 61.5 (C2′); 66.1 (C1′); 127.5–127.9–128.2–128.3–128.6–129.5–130.5 (C5, C6, C8, C9, C11, C12, C14, C15); 178.2 (C1).
IR ν (cm^{-1}): 711; 914; 1137; 1176; 1730 (C=O); 2857–3012 (–C–H); 3465 (OH). FAB+ MS (NBA): $m/z=399$ $(M+Na)^+$; 377 $(M+H)^+$; 359 (MH^+-H_2O).
25. The yield of coupling with free ethyleneglycol never exceeded 65% regardless acyl fluoride or diisopropyl carbodiimide chemistry. Acylation of mono-hydroxyl compounds with **11** proceeded in 75% and above yields.
26. Tamaru, Y.; Ochiai, H.; Sanda, F.; Yoshida, Z.-I. *Tetrahedron Lett.* **1985**, *26*, 5529.
27. Colorless oil; TLC R_f 0.13 (heptane/ethylacetate: 8/2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.89 (t, 3H, H18, $J_{H18-H17}=6.9$ Hz); 1.23–1.41 (m, 14H, H4, H5, H6, H7, H15, H16, H17); 1.53–1.64 (m, 2H, H3); 1.79–1.93 (m, 3H, H2′ and OH); 2.00–2.10 (m, 4H, H8, H14); 2.42 (t, 2H, H2, $J_{H2-H3}=7.30$ Hz); 2.55 (t, 2H, H3′, $J_{H3',H2'}=6.9$ Hz); 2.77 (t, 2H, H11, $J_{H11-H10}=J_{H11-H12}=6.5$ Hz); 3.65 (t, 2H, H1′, $J_{H1'-H2'}=6.1$ Hz); 5.29–5.43 (m, 4H, H9, H10, H12, H13). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 14.0 (C18); 22.5 (C17); 23.9 (C3); 25.6 (C11); 26.5 (C2′); 27.2 (C8, C14); 29.1 (CH_2-C); 29.2 (CH_2-C); 29.3 (CH_2-C); 29.6 (C15); 31.5 (C16); 39.5 (C3′); 42.9 (C2); 62.3 (C1′); 127.9 (C10 or C12); 128.0 (C12 or C10); 130.0 (C9 or C13); 130.2 (C13 or C9); 211.8 (C=O).
IR ν (cm^{-1}): 722; 914; 1058; 1458; 1711 (C=O); 2857–3000 (–C–H); 3416 (C–OH). FAB+ MS (NBA): $m/z=345$ $(M+Na)^+$; 323 $(M+H)^+$; 305 (MH^+-H_2O).
28. Pale yellow oil; TLC R_f 0.20 (heptane/ethylacetate: 7/3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.89 (s, 3H, CH_3); 1.21–1.51 (m, 6H, H17, H18, H19); 1.63–1.73 (m, 2H, H3); 1.80–2.04 (m, 3H, H2′, OH); 2,09–2,19 (m, 4H, H4, H16); 2.42 (t, 2H, H2, $J_{H2-H3}=7.5$ Hz); 2.55 (t, 2H, H3′, $J_{H3'-H2'}=6.9$ Hz); 2.74–3,.8 (m, 6H, H7, H10, H13); 3.4 (t, 2H, H1′, $J_{H1'-H2'}=6,09$ Hz); 5.27–5.53 (m, 8H, H5, H6, H8, H9, H11, H12, H14, H15).^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 14.2 (C20); 22.5 (C19); 23.6 (C3); 25.6 (C7, C10, C13); 27.2 (C4, C16); 29.3 (C17); 31.2 (C18); 39.5 (C3′); 42.2 (C2); 62.3 (C1′); 127.5 (CH=CH); 127.9 (CH=CH); 128.1 (CH=CH); 128.2 (CH=CH); 128.6 (CH=CH); 128.8 (CH=CH); 129.2 (CH=CH); 130.5(CH=CH); 211.4 (C=O). IR ν (cm^{-1}): 915; 1059; 1111; 1267; 1373; 1455; 1650; 1711 (C=O); 2857–3000 (C–H); 3012 (=C–H); 3418 (O–H). FAB+MS (NBA): $m/z=369$ $(M+Na)^+$; 347 $(M+H)^+$; 329 (MH^+-H_2O).