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# Synthesis of lupane triterpenoid derivatives

**Shirisha Gurrapu[1], William J.Walsh[2], James M.Brooks[2], Subash C.Jonnalagadda[2]*, Venkatram R.Mereddy[1]**

[1]Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN 55812, (USA)

[2]Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ, 08028, (USA)

E-mail: Jonnalagadda@rowan.edu

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## ABSTRACT

The synthesis of novel synthetic derivatives starting from naturally occurring lupane triterpenoid compounds has been described. Some of the key reactions involved in these syntheses include Passerini reaction, aldol reaction, and reductive amination protocols.

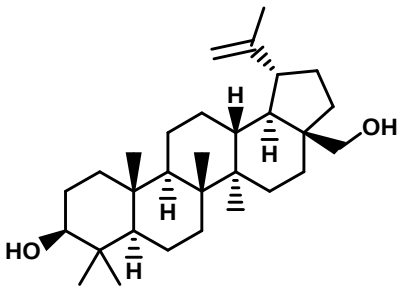


## KEYWORDS

Lupane triterpenoids;
Passerini reaction;
Aldol reaction;
Reductive amination;
Chalcones.

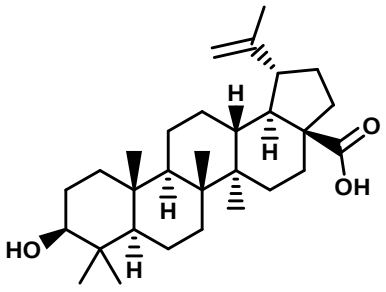
## INTRODUCTION

Betulin (1) and betulinic acid (2), (3β-hydroxy-lup-20(29)-en-28-oic acid) (Figure 1) are pentacyclic lupane triterpene natural products isolated from the bark of yellow and white birch trees[1]. Naturally, betulin occurs in >200 different types of plant species with the highest amount ranging up to 10–25% found in the outer part of birch barks[1]. These birch trees are native to northern America, and several parts of Europe. BA was found to be differentially cytotoxic against melanoma, glioblastoma, breast, prostate, and few other cancers[2]. Apart from *anti*-cancer properties, B/BA have also been reported to exhibit a wide range of biological properties such as ant-microbial, anti-HIV and anti-inflammatory activities[2]. Only a limited success has been observed in achieving high levels of anti-cancer activity for further development. Hence, there is a critical need for the design and structural modification of B/BA in order to increase the potency and broaden its biological profile against other cancers.




(1)

Betulin




(2)

Betulinic Acid

**Figure 1 : Synthesis of lupane derivatives via aldol condensation**

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In this manuscript we report the preliminary investigations of novel chemical methodologies that provide rapid access to libraries of exploratory betulin analogs, while retaining the predominant structural integrity of the parent natural products. In this regard for the present study, we chose Passerini multi-component coupling reaction[3], chalcone formation[4], and reductive amination reactions, as these protocols would produce highly flexible templates with further potential for derivatization.

## EXPERIMENTAL

### Preparation of betulin-chalcone derivative 6 via aldol condensation

To a solution of potassium tert-butoxide (6 mmol) in THF (10 mL) was added betulin aldehyde 5 (5 mmol) dissolved in 10 mL THF at 0°C and was warmed to room temperature. After stirring for 1 hour, acetophenone (6 mmol) was added and stirred overnight at room temperature. Upon completion (TLC), the reaction mixture was worked up with saturated ammonium chloride and ethyl acetate (3 x 30 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by silica gel column chromatography to obtain the betulin-chalcone derivative 6 (80% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.92 (d, *J* = 7.6 Hz, 2H), 7.44-7.56 (m, 3H), 7.34 (d, *J* = 16.4 Hz, 1H), 6.94 (d, *J* = 16.0 Hz, 1H), 4.71 (s, 1H), 4.59 (s, 1H), 3.16 (dd, *J* = 4.4, 10.8 Hz, 1H), 2.50-2.56 (m, 1H), 2.1-2.2 (br s, 1H), 0.63-2.02 (m, 24H), 1.68 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H), 0.78 (s, 3H), 0.73 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 191.3, 154.9, 149.9, 138.4, 132.9, 128.8, 128.7, 125.2, 110.4, 79.1, 55.5, 50.8, 50.6, 50.1, 47.9, 43.1, 41.0, 39.3, 39.2, 39.1, 38.9, 37.4, 34.5, 33.8, 30.0, 28.2, 28.1, 27.5, 25.5, 21.0, 19.5, 18.5, 16.3, 16.2, 15.6, 14.9; ESI-MS: 565 [$(M+Na)^+$, 100%]; 543 $(M+H)^+$.

### Preparation of betulin-chalcone-succinic acid 7

To a solution of betulin-chalcone 6 (5 mmol) in 50 mL toluene was added succinic anhydride (10 mmol), and dimethyl aminopyridine (1 mmol) and refluxed for 24 hours. Upon completion (TLC), the reaction mixture was worked up with water and ethyl acetate (3 x 25 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by silica gel column chromatography to obtain the betulin-chalcone-succinic acid derivative 7 (85% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 7.2 Hz, 2H), 7.44-7.60 (m, 3H), 7.37 (d, *J* = 15.6 Hz, 1H), 6.97 (d, *J* = 16.0 Hz, 1H), 4.75 (s, 1H), 4.63 (s, 1H), 4.51 (dd, *J* = 7.2, 8.8 Hz, 1H), 2.53-2.69 (m, 5H), 0.76-2.03 (m, 24H), 1.72 (s, 3H), 1.00 (s, 3H), 0.95 (s, 3H), 0.84 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 191.4, 178.1, 172.1, 154.9, 149.9, 138.4, 132.9, 128.8, 128.7, 125.2, 110.4, 81.8, 55.6, 50.8, 50.5, 50.1, 47.9, 43.1, 41.1, 39.3, 39.2, 38.6, 38.1, 37.3, 34.5, 33.8, 30.1, 29.6, 29.3, 28.2, 28.1, 25.5, 23.8, 21.0, 19.5, 18.4, 16.8, 16.4, 16.3, 14.9; ESI-MS: 665 [$(M+Na)^+$, 100%].

### Preparation of betulin-acetoxyamide derivative 8 via Passerini reaction

To a solution of betulin aldehyde 5 (5 mmol) in 2 mL THF was added benzoic acid (5.5 mmol) and isopropyl isocyanide (5.5 mmol) and stirred for 72 hours at room temperature. Upon completion (TLC), the reaction mixture was worked up with water and ethyl acetate (3 x 20 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by silica gel column chromatography to obtain the betulin-acetoxyamide derivative 8 (70% yield) as a single diastereomer. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.06-8.09 (m, 2H), 7.41-7.64 (m, 3H), 5.82 (s, 1H), 5.70 (d, *J* = 8.0 Hz, 1H), 4.51 (s, 1H), 4.48 (s, 1H), 4.44 (dd, *J* = 5.2, 10.8 Hz, 1H), 4.01-4.09 (m, 1H), 2.64-2.71 (m, 1H), 0.74-2.33 (m, 24H), 2.00 (s, 3H), 1.65 (s, 3H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.09 (s, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.98 (s, 3H), 0.82 (s, 3H), 0.81 (s, 3H), 0.80 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 171.3, 168.7, 165.7, 150.6, 133.9, 129.8, 129.6, 129.1, 110.0, 81.2, 77.5, 76.3, 55.6, 50.7, 50.4, 49.4, 48.6, 43.2, 41.1, 38.6, 38.0, 37.3, 37.2, 35.0, 34.4, 33.7, 32.6, 28.3, 28.2, 25.3, 23.9, 22.9, 22.7, 21.6, 21.0, 19.0, 18.4, 16.7, 16.4, 16.3, 15.4; ESI-MS: 696 [$(M+Na)^+$, 100%].

### Preparation of betulin acetoxyamide derivative 9 via Passerini reaction

Procedure similar to the preparation of 8 above. Benzyl isocyanide was used instead of isopropyl isocyanide. 75% yield of the product betulin acetoxyamide 9 was obtained. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.05-8.07 (m, 2H), 7.44-7.64 (m, 4H), 7.16-7.27 (m, 4H),

6.34 (t, *J* = 6.0 Hz, 1H), 5.96 (s, 1H), 4.54 (s, 1H), 4.51 (s, 1H), 4.39-4.48 (m, 3H), 2.66-2.74 (m, 1H), 0.74-2.37 (m, 24H), 2.02 (s, 3H), 1.67 (s, 3H), 1.11 (s, 3H), 1.01 (s, 3H), 0.84 (s, 3H), 0.83 (s, 3H), 0.82 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 171.4, 169.7, 165.9, 150.6, 138.2, 133.9, 129.9, 129.6, 129.0, 128.9 128.6, 127.6, 110.1, 81.2, 77.5, 76.5, 55.6, 50.8, 50.4, 49.4, 48.7, 43.3, 41.1, 38.6, 38.0, 37.4, 37.2, 35.1, 34.4, 33.7, 32.6, 28.4, 28.2, 25.3, 23.9, 21.5, 21.0, 19.0, 18.4, 16.7, 16.4, 15.5, 14.4; ESI-MS: 744 [$(M+Na)^+$, 100%].

**Preparation of betulin-BODIPY-acetoxyamide derivative 11 via Passerini reaction**

Procedure similar to the preparation of 8 above. *p*-BODIPY-benzoic acid 10 was used instead of benzoic acid. 72% yield of the product betulin acetoxyamide 9 was obtained. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.22-7.28 (m, 5H), 6.34 (t, *J* = 5.6 Hz, 1H), 5.85 (s, 1H), 4.67 (s, 1H), 4.58-4.64 (m, 2H), 4.45-4.50 (m, 1H), 4.35 (dd, *J* = 4.8, 10.4 Hz, 1H), 2.78-2.87 (m, 1H), 2.52 (s, 6H), 0.74-2.37 (m, 24H), 2.28 (q, *J* = 7.4 Hz, 4H), 2.03 (s, 3H), 1.68 (s, 3H), 1.22 (s, 6H), 1.15 (s, 3H), 1.01 (s, 3H), 0.97 (t, *J* = 7.4 Hz, 6H), 0.84 (s, 3H), 0.83 (s, 3H), 0.82 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 171.3, 170.6, 165.2, 154.6, 150.2, 141.7, 138.5, 138.2, 137.8, 133.4, 130.6, 130.4, 129.9, 129.3, 128.9, 128.0, 127.9, 110.4, 81.2, 77.5, 75.0, 55.6, 52.5, 50.5, 50.2, 46.8, 43.2, 41.2, 38.6, 38.0, 37.3, 34.6, 34.3, 31.8, 31.0, 29.2, 28.2, 25.3, 23.9, 22.9, 21.5, 21.0, 19.0, 18.4, 17.3, 16.6, 16.3, 15.4, 14.8, 14.3, 12.8, 12.2; ESI-MS: 1046 [$(M+Na)^+$, 100%].

**Preparation of betulin *N*-benzyl amine 13 via reductive amination**

To a solution of betulin aldehyde 12 (5 mmol) in toluene (20 mL) was added benzyl amine (6 mmol) and refluxed for 6 hours. The reaction mixture was cooled to room temperature, and solvent was concentrated *in vacuo*. The crude reaction mixture was dissolved in methanol (10 mL) and added slowly to a suspension of $NaBH_4$ (3 mmol) in methanol (5 mL) under inert atmosphere at 0°C. The reaction mixture was stirred overnight at room temperature. Upon completion (TLC), the reaction mixture was concentrated *in vacuo* and worked up with water and ethyl acetate (3 x 30 mL). The combined organic layers were dried ($MgSO_4$), concentrated *in vacuo* and purified by silica gel column chromatography to obtain the betulin *N*-benzyl amine derivative 13 (82% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.23-7.36 (m, 5H), 4.64 (s, 1H), 4.54-4.55 (m, 1H), 3.91 (d, *J* = 13.6 Hz, 1H), 3.78 (d, *J* = 13.2 Hz, 1H), 3.16 (dd, *J* = 5.2, 11.2 Hz, 1H), 2.68 (d, *J* = 11.2 Hz, 1H), 2.32-2.39 (m, 1H), 2.19 (d, *J* = 11.2 Hz, 1H), 0.64-1.93 (m, 24H), 1.66 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.81 (s, 3H), 0.80 (s, 3H), 0.75 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 150.9, 128.6, 128.5, 128.3, 127.2, 109.7, 79.2, 77.5, 55.5, 54.7, 50.6, 49.5, 47.7, 46.8, 42.7, 41.0, 39.1, 38.9, 37.3, 37.2, 35.4, 34.4, 30.5, 30.2, 28.2, 27.6, 27.3, 25.3, 21.0, 19.4, 18.5, 16.3, 15.9, 15.6, 15.0; ESI-MS: 532 [$(M+H)^+$, 100%].

**Preparation of betulin N,N-Dibenzyl amine 14**

To a solution of betulin *N*-benzyl amine 13 (5 mmol) in $CH_2Cl_2$ (10 mmol) was added benzyl bromide (6 mmol) and N,N-diisopropylethyl amine (6 mmol) and stirred for 24 hours. Upon completion (TLC), the reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography to obtain the betulin- *N*,*N*-Dibenzyl amine derivative 14 (78% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.22-7.40 (m, 10H), 4.61 (s, 1H), 4.52-4.53 (m, 1H), 3.61 (d, *J* = 13.6 Hz, 2H), 3.53 (d, *J* = 13.6 Hz, 2H), 3.17 (dd, *J* = 4.8, 11.2 Hz, 1H), 2.65 (d, *J* = 13.6 Hz, 1H), 2.20-2.27 (m, 2H), 1.95-2.04 (m, 2H), 0.66-1.73 (m, 23H), 1.63 (s, 3H), 0.96 (s, 3H), 0.91 (s, 6H), 0.82 (s, 3H), 0.77 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): δ 151.2, 140.2, 129.4, 128.4, 127.1, 109.5, 79.2, 77.5, 61.2, 55.5, 51.6, 50.6, 50.5, 47.7, 42.7, 41.1, 39.1, 38.9, 37.3, 37.0, 36.1, 34.4, 31.9, 30.1, 28.2, 27.6, 27.3, 25.4, 21.0, 19.4, 18.5, 16.4, 15.9, 15.6, 14.9; ESI-MS: 622 [$(M+H)^+$, 100%].

## RESULTS AND DISCUSSION

We initiated our project with the isolation of the natural product. Betulin was isolated from birch bark by a simple extraction process. The birch wood was either obtained from local birch trees or purchased from grocery store (commonly available as firewood). The bark was peeled off the log using a knife, and chopped

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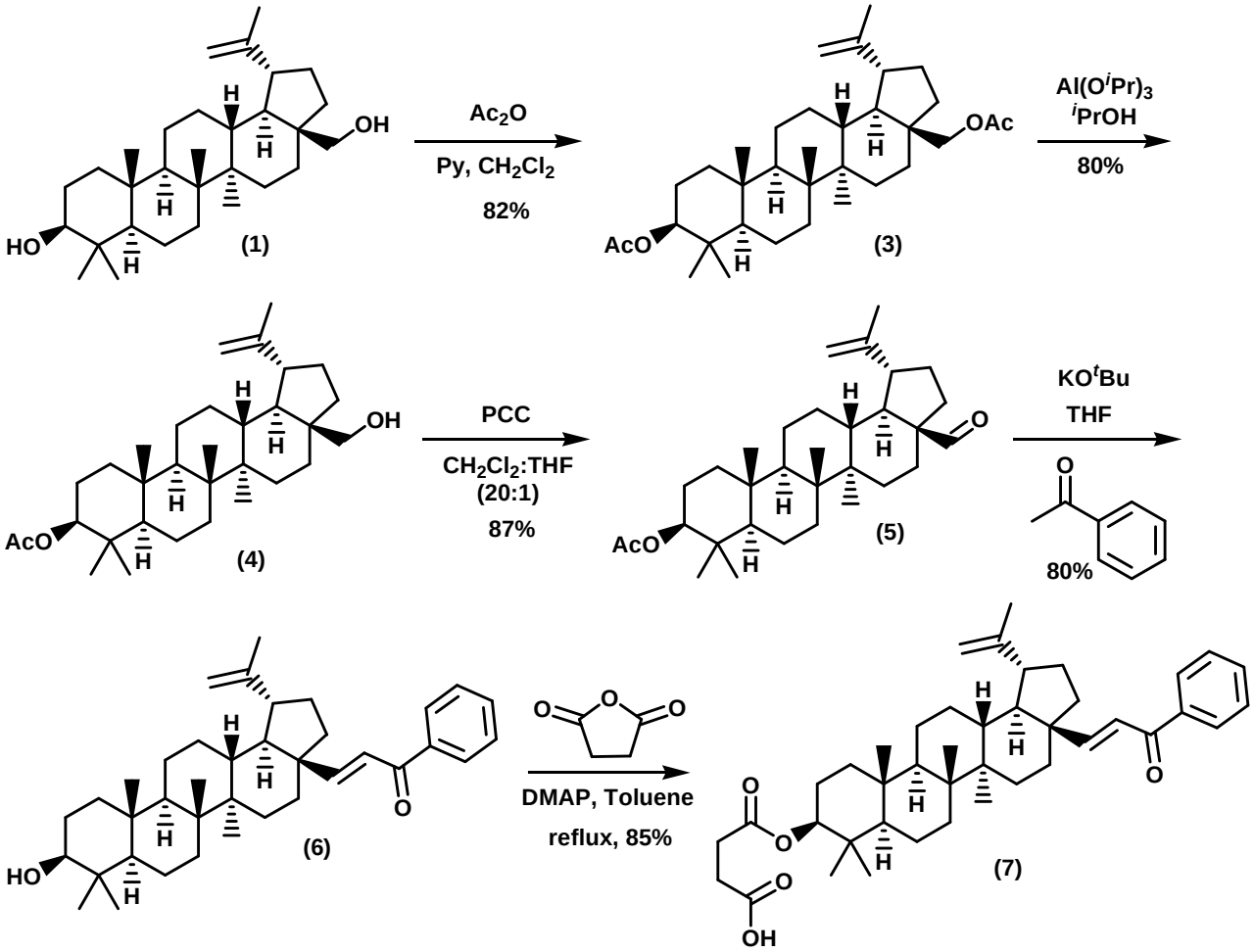
in to smaller pieces. The pieces were ground in a regular kitchen blender and refluxed in chloroform for 4-6 hours. The mixture was filtered on a sintered funnel and the extract evaporated on a rotary evaporator to obtain crude betulin. Recrystallization in ethanol afforded pure betulin as an off white powder. We have been able to routinely isolate multiple grams (~30-40g) of betulin with utmost ease using this procedure.

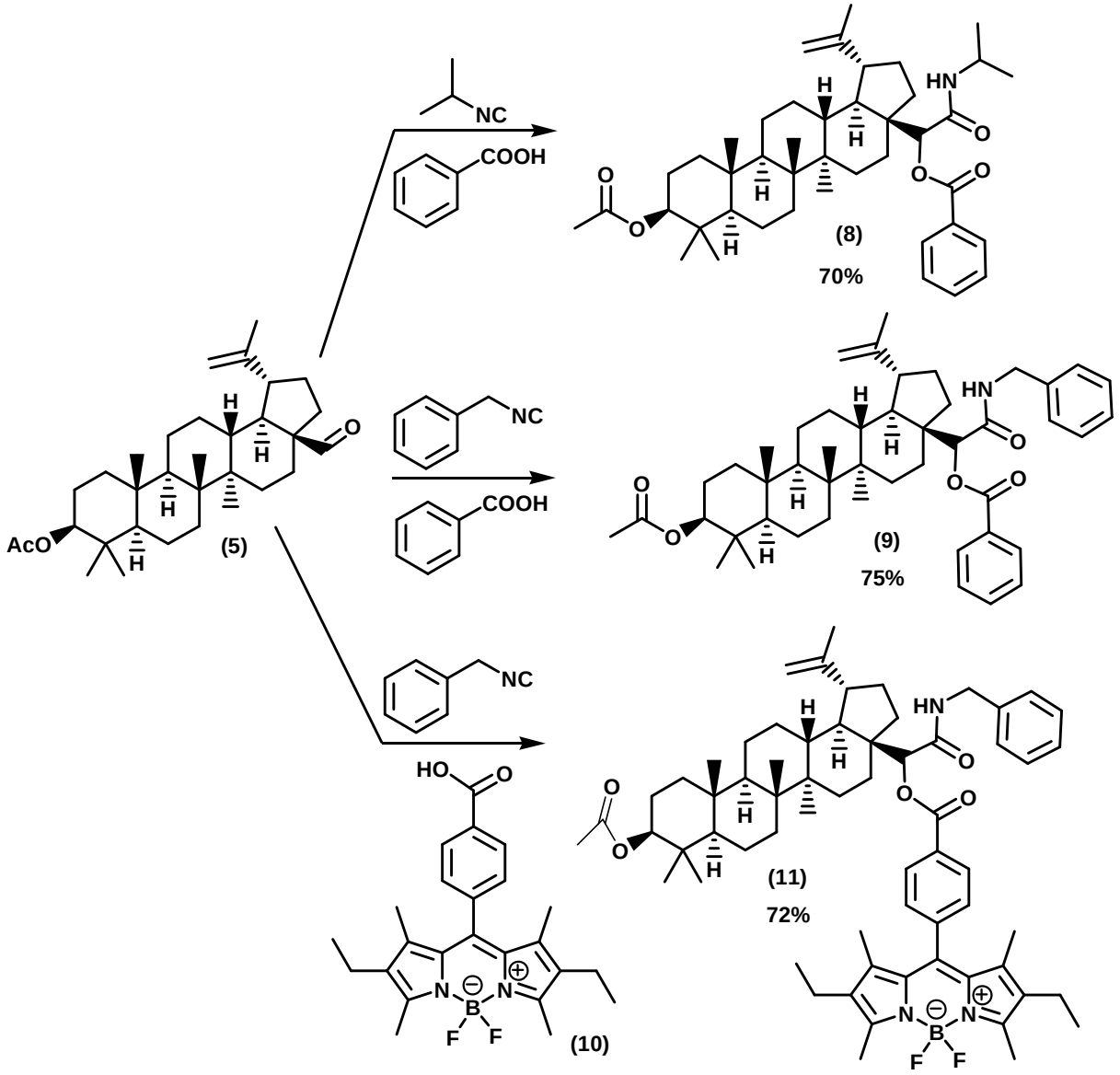
Owing to the importance of chalcones as *anti*-cancer pharmacophores in medicinal chemistry[4] we synthesized chalcone hybrid analogs of Betulin 6-7 as shown in Scheme 1. The synthesis of chalcone derivatives was initiated with the synthesis of betulin aldehyde 5. Acetylation of betulin 1 with acetic anhydride provided the betulin diacetate 3, which upon regioselective hydrolysis of primary acetate with aluminum isopropoxide furnished betulin hydroxyacetate 4. PCC oxidation of the resulting $C_{28}$ primary alcohol in 4 furnished betulin aldehyde 5 in 57% overall yield over three steps[5]. Condensation of aldehyde 5 with acetophenone using KO$^t$Bu furnished the α-β-unsaturated ketone 6 upon concomitant hydrolysis of the $C_3$ acetate group under aldol condensation conditions. To improve the water solubility of this compound, the $C_3$ secondary alcohol was converted into succinic acid hemiester 7 by refluxing the ketone 6 with succinic anhydride in toluene (Scheme 1).




**Scheme 1 : Synthesis of lupane derivatives via aldol condensation**

Multi-component coupling is an extremely important tool in organic and medicinal chemistry towards the synthesis of structurally diverse scaffolds of biological interest. The isocyanide based Passerini and Ugi coupling reactions offer an easy access to a diverse range of peptidomimetic analogs under mild reaction conditions[3]. Accordingly, we utilized betulin aldehyde 5 in the Passerini reaction via coupling with benzoic acid in the presence of isopropyl and benzyl isonitriles to afford the corresponding α-acetoxyamides in good yield and diastereoselectivity. The high selectivity observed in the reaction could be attributed to

the substrate controlled nucleophilic addition from the side opposite to that of the bulky pivalyl group. Similarly, a fluorescent derivative of betulin was also synthesized utilizing Passerini reaction of betulin aldehyde 5 with BODIPY acid 10[6] and benzyl isocyanide (Scheme 2).




**Scheme 2 : Synthesis of lupane derivatives via Passerini multicomponent coupling**

In another protocol, betulin aldehyde 12 was synthesized directly from betulin via PCC oxidation and further subjected to reductive amination with benzyl amine in the presence of sodium borohydride to prepare the betulin N-benzylamine 13. The amine 13 was further utilized for nucleophilic substitution on benzyl bromide to prepare betulin N,N-dibenzyl amine derivative 14 in high yield. These amines were prepared in an effort towards increasing the water solubility of these betulin compounds (Scheme 3).

In conclusion, we have prepared several synthetic derivatives of lupane triterpenoids employing reactions such as Passerini multicomponent coupling reaction, aldol reaction, and reductive amination reaction. The biological evaluation of these synthetic derivatives as potential anti-cancer agents is in progress and will be reported in due course. Owing to the importance of natural products in medicinal chemistry, the present manuscript would find interest amongst the synthetic organic chemistry community.

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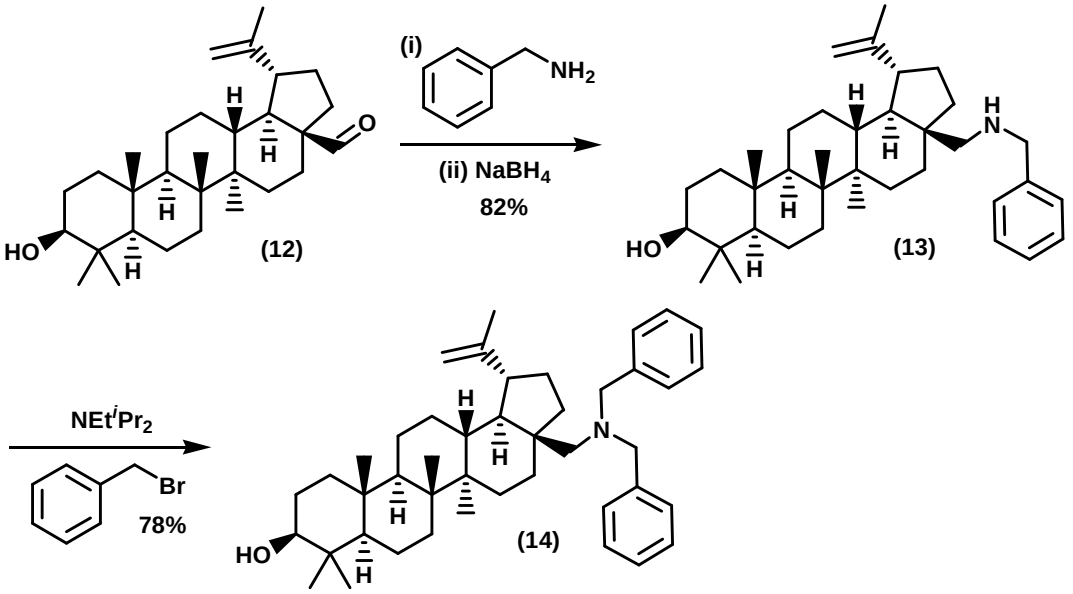


**Scheme 3 : Synthesis of lupane derivatives via reductive amination protocol**


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## REFERENCES


**[1]** **(a)** T.Galgon, D.Hoke, B.Drager; Phytochem.Anal., **10**, 187-190 **(1999)**; **(b)** P.A.Krasutsky; Nat.Prod.Rep., **23**, 919-942 **(2006)**.

**[2]** **(a)** S.Fulda; Int.J.Mol.Sci., **9**, 1096-1107 **(2008)**; **(b)** S.Chintharlapalli, S.Papineni, S.K.Ramaiah, S.Safe; Cancer Res., **67**, 2816-2823 **(2007)**; **(c)** G.R.Jung, K.J.Kim, C.H.Choi, T.B.Lee, S.I.Han, H.K.Han, S.C.Lim; Basic Clin.Pharmacol.Toxicol., **101**, 277-285 **(2007)**; **(d)** E.Pisha, H.Chai, I.S.Lee, T.E.Chagwedera, N.R.Farnsworth, G.A.Cordell, C.W.W.Beecher, H.H.S.Fong, A.D.Kinghorn, D.M.Brown, M.C.Wani, M.E.Wall, T.J.Hieken, T.K.Dasgupta, J.M.Pezzuto; Nature Med., **1**, 1046-1051 **(1995)**.

**[3]** **(a)** C.Hulme; Multicomponent Reactions, 311-341 **(2005)**; **(b)** A.Domling, I.Ugi; Angew Chem.Int.Ed., **39**, 3168-3210 **(2000)**; **(c)** L.Banfi, R.Riva; Organic Reactions, **65**, 1-141 **(2005)**.

**[4]** **(a)** T.H.Kim, W.D.Seo, H.W.Ryu, H.R.Seo, Y.B.Jin, M.Lee, Y.H.Ji, K.H.Park, Y.S.Lee; Chemico-Biological Interactions, **188**, 111-118 **(2010)**; **(b)** E.Szliszka, Z.P.Czuba, B.Mazur, A.Paradysz, W.Krol; Molecules, **15**, 5336-5353 **(2010)**; **(c)** M.Cabrera, M.L.Lavaggi, F.Croce, L.Celano, L.Thomson, M.Fernández, C.Pintos, S.Raymondo, M.Bollati, A.Monge, A.L.D.Ceráin, O.E.Piro, H.Cerecetto, M.González; Bioorg.Med.Chem., **18**, 5391-5394 **(2010)**; **(e)** A.Sharma, B.Chakravarti, M.P.Gupt, J.A.Siddiqui, R.Konwar, R.P.Tripathi; Bioorg.Med.Chem., **18**, 4711-4720 **(2010)**.

**[5]** D.Thibeault, C.Gauthier, J.Legault, J.Bouchard, P.Dufour, A.Pichette; Bioorg.Med.Chem., **15**, 6144-6157 **(2007)**.

**[6]** M.Tomasulo, E.Deniz, R.J.Alvarado, F.M.Raymo; J.Phys.Chem.C, **112**, 8038-8045 **(2008)**.