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Efficient Synthesis of the Insect Elicitor Volicitin and Biologically Active Analogs

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This paper is dedicated to Professor Tom J. Mabry for his 75th birthday.

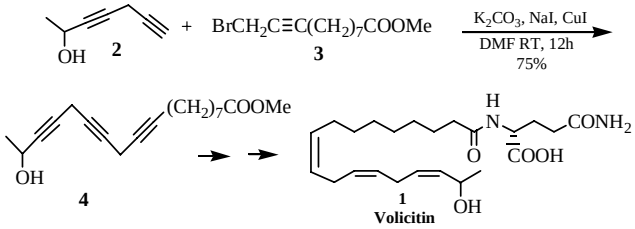
An improved and efficient copper (I) mediated coupling procedure was used to synthesize *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin), a chemical elicitor from the herbivore pest beet army worm, and its biologically active analogs.

Keywords: *N*-(17*S*-hydroxylinolenoyl)-L-glutamine, volicitin, synthesis, analog.

Biotic elicitors derived from either plant pathogens or herbivore pests rapidly trigger a range of chemical defenses in plants that are not mimicked by mechanical damage alone [1]. Three structurally similar amides of linolenic acid, *N*-(17*S*-hydroxylinolenoyl)-L-glutamine (volicitin) (**1**), *N*-linolenoyl-L-glutamine and *N*-linolenoyl-L-glutamic acid are responsible for a majority of elicitor activity associated with Lepidopteran larvae [2-4].

Volicitin analogs in which linolenic acid is substituted for linoleic acid or glutamine (Gln) or the hydroxyl unit is removed results in a loss of elicitor activity in triggering plant defense responses [5]. Substitution of D-Gln for the natural L-form reduces volicitin's biological activity by greater than 90%, suggesting a ligand-receptor type interaction. Using filter binding assays, a maize plasma membrane binding protein has been identified for volicitin. To tag volicitin binding proteins, a versatile synthetic strategy has been devised for the assembly of volicitin analogs that can covalently bind *via* photoactive azide units. Primary amines provide a reactive site in either coupling azide tags or act as linkers for coupling to a resin. Volicitin analogs can also be used to investigate the physical restraints of ligand-receptor binding.

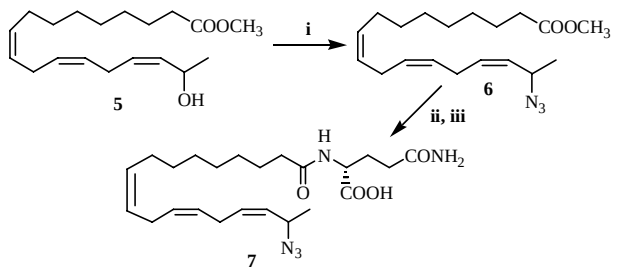
Several synthetic routes for generating the 17-hydroxylinolenoyl skeleton have been reported, albeit such schemes have exhibited limited versatility in generating diverse structural analogs of volicitin [6,7]. We present here an efficient and convenient protocol for the synthesis of the 17-hydroxylinolenate skeleton in gram quantities based on the copper (I) mediated cross coupling of 3,6-heptadiyn-2-ol with propargylic bromides. The overall yield for the four step procedure producing volicitin is ca. 50%. The same coupling protocol is employed to generate modified volicitin derivatives *viz.* *N*-(17-azidolinolenoyl)-L-glutamine and *N*-(8-amino-17-hydroxylinolenoyl)-L-glutamine hydrochloride. The key step in the synthetic strategy was the coupling of diynol (**2**) with propargylic bromide (**3**) to form the C-17 triynol (**4**) (Scheme 1).



Scheme 1: Modified synthesis of volicitin (**1**).

The most common coupling approach for forming such a methylene-interrupted polyacetylene carbon chain is based on cuprous chloride-catalyzed Grignard coupling of a protected diynol with a unit containing propargylic bromide [8,9], or similar methods [10]. However, we found that such protocols were not well suited for gram-scale synthesis. The elevated temperatures (~ 55°C) and harsh conditions used in the Grignard reaction for the key coupling reaction further served to lower the yields of the C-17 triynol product (**4**). In order to circumvent these problems and to synthesize volicitin and its analogs with higher yields, we have developed a strategy using copper (I) mediated cross coupling of diynols and propargyl bromides. The procedure reported here is also more versatile for the generation of volicitin analogs. A convergent strategy connects a diynol (**2**) and propargylic bromide (**3**) to form the methylene interrupted polyacetylene carbon backbone (Scheme 1).

This copper (I) mediated cross coupling generated C-17 triynol (**4**) in over 75% yield. Since the reaction was carried out at room temperature and under mild conditions, degradation of the sensitive C-17 triynol was prevented. The diynol unit (**2**) was prepared with an overall yield of 50% from (±)-3-butyn-2-ol in three steps through a Grignard coupling of the corresponding TBS protected alcohol and propargyl bromide. The propargylic unit (**3**) has been prepared earlier in three steps from commercially available 8-bromooctanoic acid [7]. As **4** has been elaborated to volicitin earlier in our laboratory, this constitutes the formal synthesis of *N*-(17*RS*-hydroxylinolinenoyl)-L-glutamine with an overall yield of 50% from intermediates **2** and **3**. By directly using a C-11 propargyl bromide instead of a previously published propargyl iodide [7] the total number of steps was reduced and by using mild and efficient coupling conditions the overall yield was increased.



Scheme2: Synthesis of azido substituted volicitin analog *N*-(17-azidolinolenoyl)-L-glutamine (**7**).
i) PPh_3-DEAD-DPPA/THF, 75% ii) LiOH/THF-H_2O iii) ClCOOEt/NEt_3/Glutamine, 80% , 2 steps

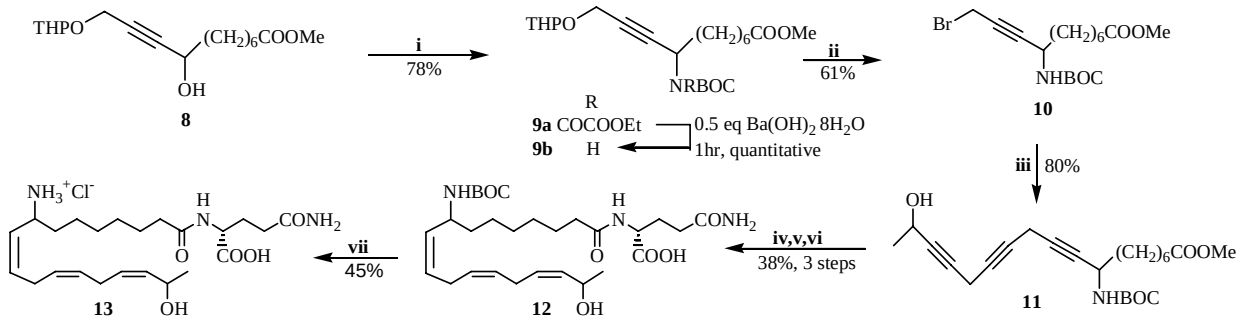
N-(17-Azidolinolenoyl)-L-glutamine was prepared from the (*Z*,*Z*,*Z*)-trienol intermediate (**5**) in three steps with a overall yield of approximately 60% (Scheme 2). 17-Hydroxylinolenoate (**5**) can be readily prepared by P2-Ni reduction of **4** [7]. The C-17 hydroxy group in **5** was converted to the corresponding azide under Mitsunobu conditions using diphenylphosphoryl azide [11]. The resulting 17-azidolinolenoate (**6**) was hydrolyzed and coupled with glutamine to afford **7**.

The strategy for the synthesis of *N*-(8-amino-17-hydroxylinolenoyl)-L-glutamine mandated the introduction of nitrogen at the beginning of the synthetic scheme. Consequently, the known C-8 propargylic THP ether (**8**) [12] was chosen as the starting material. The protected amine was introduced using *N*-Boc ethyloxamate as the amine equivalent under Mitsunobu conditions (Scheme 3) [13]. Barium hydroxide mediated partial hydrolysis of **9a**, followed by bromination of the propargylic ether (**9b**) under triphenyl phosphine/bromine conditions, led to the propargylic bromide (**10**). Intermediates **10** and **2** underwent the copper (I) mediated coupling to give the C-8 substituted triyne ester (**11**).

Catalytic hydrogenation of **11**, first with P_2-Ni [14] followed by Pd-$BaSO_4$ as the catalyst, led to the nitrogen substituted C-18 triene intermediate, which was then hydrolyzed and coupled with glutamine to yield the protected *N*-(8-amino-17-hydroxy-linolenoyl)-L-glutamine (**12**) in an overall yield of approximately 30% from the C-8 substituted propargylic bromide (**10**). Removal of the *t*-butoxy carbonyl using dry HCl afforded *N*-(8-amino-17-hydroxy-linolenoyl)-L-glutamine hydrochloride (**13**).

Elicitor Activity

In order to probe plant induction responses by volicitin and its analogs, the sum of emissions of the major maize volatile components were measured [15]. Injection of either volicitin or the synthetic analogue 17-N_3-volicitin (**7**) into maize seedlings triggered the emission of approximately the same quantity of volatile terpenes and indole, while the volicitin analogue *N*-(8-*N*-Boc-amino-17-hydroxylinolenoyl)-L-glutamine (**12**) generated close to a ten-fold lower level of terpene emissions (Figure 1). Surprisingly, *N*-(8-amino-17-hydroxylinolenoyl)-L-glutamine hydrochloride (**13**) showed almost no increase of volatiles compared to control buffer conditions. If these three different analogs of volicitin are entering into the same binding pocket of the



Scheme 3: Synthesis of C-8 amino substituted volicitin analog *N*-(8-amino-17-hydroxylinolenoyl)-L-glutamine hydrochloride. i) PPh_3/DEAD/*N*-BOC-Ethyloxamate/THF, 78% ii) PPh_3-Br_2/CH_3CN, -10ºC, 2h, 61% iii) Unit A; NaI/K_2CO_3/DMF, 80% iv) P2-Ni/H_2 & Pd-$BaSO_4$/H_2 v) LiOH (THF-H_2O) vi) ClCOOEt/Net_3/Glutamine, 38%, 3 steps vii) Dry HCl-EtOAc, 45%.

volicitin binding protein to trigger volatile emissions, it is most likely that the bulky 8-*N*-BOC-amino unit and the highly polar 8-amine hydrochloride unit located in the center of the volicitin backbone interferes with effective ligand-protein binding, while an azido group substitution for the original hydroxyl unit in the hydrocarbon tail region does not prevent effective binding.

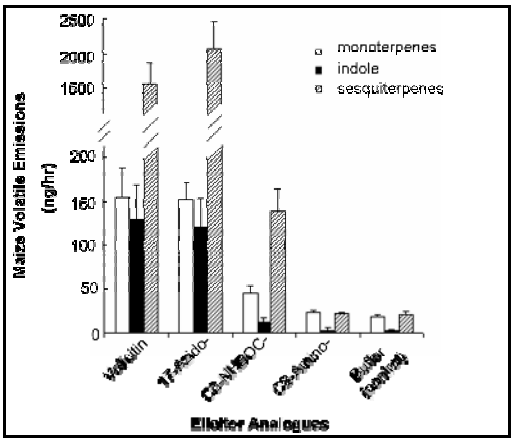


Figure 1: Volatile emissions triggered by volicitin and its analogs.

In conclusion, a concise and high yielding procedure for the synthesis of the insect elicitor volicitin is reported. In addition, two biologically active analogs of volicitin containing strategically placed nitrogen substituents have been synthesized based on the copper (I) mediated cross coupling of diynes with C-8 propargylic bromides. Finally, this efficient cross coupling methodology can be conveniently applied to prepare other analogs of volicitin.

Experimental

General procedure for the coupling of propargylic bromides with diynol: To a stirred suspension of dry K_2CO_3 (1.5eq), sodium iodide (2eq) and copper (I) iodide (2eq) in dry DMF under nitrogen, a solution of the propargylic bromide (1eq/**3** or **10**) in dry DMF was added. This was followed by the addition of the diynol (**2**) (1.05eq) in dry DMF. After stirring for 12 hours at room temperature, water was added to the reaction followed by extraction with either ethyl ether or ethyl acetate. The organic phase was washed with brine, dried and concentrated to afford the corresponding C-18 triynol.

All proton and carbon assignments are based on DEPT, HMQC and HMBC experiments.

3,6-Heptadiyn-2-ol (2) [7]

^{1}H NMR (500 MHz; $CDCl_3$) δ: 1.42 (3H, d, *J* = 8 Hz, H-1), 2.06 (1H, t, *J* = 3 Hz, H-7), 3.18 (2H, Ap t, *J* = 2 Hz, H-5), 4.50 (1H, tq, *J* = 8, 2 Hz, H-2).
^{13}C NMR (125 MHz, $CDCl_3$) δ: 9.5 (C-5), 24.1 (C-1), 58.2 (C-2), 68.8 (C-7), 73.8, 77.9, 82.7

Methyl 11-bromo-8-(*t*-butoxycarbonylamino)-9-undecynoate (10)

^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.27-1.29 (4H, m, H-4, H-5), 1.35-1.39 (11H, m, *t*-butyl, H-6), 1.54-1.60 (4H, m, H-3, H-7), 2.26 (2H, t, *J* = 8 Hz, H-2), 3.62 (3H, s, $COOCH_3$), 3.87 (2H, d, *J* = 2 Hz, CH_2Br), 4.39 (1H, br s, H-8), 4.76 (1H, br s, *NH*BOC).
^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.3 (CH_2Br), 24.6 (C-7), 25.2 (C-6), 28.2 (CH_3, *t*-butyl), 28.5 (C-4/C-5), 28.8 (C-5/C-4), 33.9 (C-2), 35.8 (C-3), 42.8 (C-8), 51.3 ($COOCH_3$), 77.8 (C≡C), 79.7 ($OC(CH_3)_3$, BOC), 86.5 (C≡C), 154.7 (C=O, BOC), 174.1 ($COOCH_3$).
APCI-MS (Positive) : m/z = 390.7 [M], 334.8 [M-56], 291.06 [334.8-44].

***N*-(17-Azidolinolenoyl)-L-glutamine (7)**

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.16 (3H, d, J = 7 Hz, H-18), 1.19-1.34 (8H, m, H-4, H-5, H-6, H-7), 1.41-1.49 (2H, m, H-3), 1.70-1.75 (1H, m, CH_2, Glu), 1.88-1.95 (1H, m, CH_2, Glu), 1.98-2.03 (2H, m, H-8), 2.07-2.11 (4H, m, H-2, CH_2, Glu), 2.72-2.82 (4H, m, H-11, H-14), 4.08-4.15 (2H, m, H-17, CH Glu), 5.26-5.47 (5H, m), 5.69-5.79 (1H, m,), 6.75 (1H, s, NH_2), 7.27 (1H, s, NH_2), 8.02 (1H, d, J = 8 Hz, NH), 12.49 (1H, br s, COOH).
^{13}C NMR (125 MHz, DMSO-d_6) δ: 19.8 (C-18), 25.26 (C-3), 25.43 (C-11), 26.67 (C-8), 26.88 (CH_2, Glu), 28.63 (CH_2), 28.66 (CH_2), 28.75 (CH_2), 29.07 (CH_2), 29.38 (CH_2), 31.41 (CH_2, Glu), 35.09 (C-2), 51.50 (CH, Glu), 58.70 (C-17), 126.59 (C-12/C-13), 127.31 (C-10), 129.07 (C-13/C-12), 129.83 (C-15/C-16), 130.05 (C-9), 131.74 (C-16/C-15), 172.33 (C-1), 173.48 ($CONH_2$, Glu), 173.63 (COOH, Glu)
APCI MS (Negative mode): m/z = 446.2 [M-1], MS-MS: m/z = 403.2

***N*-(8-*N*-*t*-Butoxycarbonylamino-17-hydroxylinolenoyl)-L-glutamine (12)**

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.05-1.08 (3H, d, J = 7 Hz, H-18), 1.20-1.52 (19 H, m, H-3, H-4, H-5, H-6, H-7, *t*-butyl), 1.67-1.75 (1H, m, CH_2, Glu), 1.87-1.94 (1H, m, CH_2, Glu), 2.06-2.11 (4 H, m, H-2, CH_2, Glu), 2.73-2.80 (2H, m, H-11/H-14), 2.92-2.98 (2H, m, H-14/H-11), 4.09-4.20 (2H, m, H-8, CH, Glu), 4.39-4.49 (1H, m, H-17), 5.17-5.43 (6H, m, olefinic), 6.76 (1H, s, NH_2, Glu), 7.27 (1H, s, NH_2, Glu), 8.03 (1H, d, J = 8 Hz, NH, Glu).
^{13}C NMR (125 MHz, DMSO-d_6) δ: 16.5 (C-11/C-14), 24.1 (C-18), 25.1 (C-3), 25.4 (C-11/C-14 & C-4/C-5/C-6), 26.8 (β-CH_2, Glu), 28.2 (CH_3, *t*-BOC), 28.3 (C-4/C-5/C-6), 28.6 (C-4/C-5/C-6), 31.4 (γ-CH_2, Glu), 35.1 (CH_2, C-2), 35.5 (C-7), 47.2 (CH, C-8), 51.5 (CH, Glu), 62.1 (CH, C-17), 77.4 (O*C*(CH_3)$_3$, BOC), 126.1 (CH), 127.9 (CH), 128.0 (CH), 128.9 (CH), 130.2 (CH), 135.9 (CH), 154.8 (C=O, BOC), 172.3 (C=O), 173.4 (C=O), 173.6 (C=O).
APCI MS (Negative): m/z = 536.2 (M-1); APCI MS-MS: m/z = 462.2, 444.2, 419.2.

***N*-(8-Amino-17-hydroxylinolenoyl)-L-glutamine hydrochloride (13)**

^{1}H NMR (500 MHz, D_2O) δ: 0.98-1.16 (7H, m, H-18, H-4, H-5), 1.20-1.28 (2 H, m, H-6), 1.32-1.39 (2H, m, H-3), 1.50-1.58 (2H, m, H-7), 1.74-1.82 (1H, m, β-CH_2, Glu), 1.92-1.99 (1H, m, β-CH_2, Glu), 2.08 (2H, t, J = 7 Hz, H-2), 2.16 (2H, t, J = 7 Hz, γ-CH_2, Glu), 2.56-2.60 (2H, m, H-11/H-14), 2.79-2.94 (2H, m, H-14/H-11), 3.82-3.92 (1H, m, H-8), 4.02-4.06 (1H, m, H-17), 5.20-5.70 (6H, m, olefinic).
^{13}C NMR (125 MHz, D_2O) δ: 16.7 (C-11/C-14), 22.5 (C-18), 24.9 (C-6), 25.3 (C-3), 26.8 (β-CH_2, Glu), 27.9 (C-4/C-5), 28.2 (C-5/C-4), 31.2 (γ-CH_2, Glu), 33.3 (C-7), 35.6 (C-2), 43.6 (C-8), 52.4 (CH, Glu), 68.5 (C-17), 124.9 (CH), 126.2 (CH), 128.6 (CH), 129.1 (CH), 130.2 (CH), 132.5 (CH), 175.3 (C=O), 177.5 (C=O), 178.2 (C=O).
ESI MS (Negative): m/z = 472 [M-H], 436 [M-HCl]; ESI MS-MS: m/z = 418 [436-H_2O], 145 [Glu]

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