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# Synthesis of a polyprenyl-type library containing 1,4-disubstituted-1,2,3-triazoles with anti-biofilm activities against *Pseudoalteromonas* sp.

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ABSTRACT

A terpenoid-like library containing 1,4-disubstituted-(1*H*)-1,2,3-triazoles was prepared by means of 1,3-dipolar cycloaddition of geranyl and farnesyl azides with a set of terminal alkynes, in order to design a new class of potentially active anti-biofilm compounds. Reactions were optimized to proceed under mild conditions and in high yields. Two compounds were found to possess interesting activity against *Pseudoalteromonas* sp. biofilm. This process is suitable for combinatorial chemistry of marine natural product-like compounds.



Biofilms are agglomerates of bacteria on a surface that is surrounded or held together by extracellular polymeric substances. Bacteria produce these, in part, to help them attach to surfaces and bind to one another. Such biofilms cause persistent infections in humans and serious corrosion and equipment failure in industrial settings.[1] In continuation of our program aimed at establishing the potentiality of marine natural products as potential antifouling compounds,[2] our group recently isolated a series of meroditerpenes from brown alga *Halidrys siliquosa*.[3] These compounds were derivatives of geranylgeranyltoluquinol and represent an interesting class of biologically active compounds exhibiting antifouling properties against marine bacteria. In addition, meroditerpenes were also found in diverse marine organisms such as brown alga *Cystoseira crinita*,[4] and were reported to possess anticancer and anti-oxidant activities. Furthermore, 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid isolated from *Acronychia baueri* Schott[5] was reported for its capacity to inhibit the formation of *P. gingivalis* biofilm. In an effort to probe antifouling structure–activity relationships of natural products, we felt that the relatively simple structure of these compounds invited the synthesis of analogs. In this context, we needed to develop a rapid and efficient synthesis of libraries of analogs. The general structure of the targeted library, as well as the retrosynthetic analysis is presented in Figure 1.

Conjugation of the aromatic moiety to the lipophilic chain was planned to be achieved by click reaction between two different azido derivatives of terpenes and aromatic-alkyne derivatives. The copper(I)-catalyzed 1,3-dipolar cycloaddition of organic azides and alkynes resulting in the formation of 1,2,3-triazoles has become an increasingly attractive area because it is a highly efficient process in bond formations among diverse building blocks for combinatorial chemistry.[6] In addition, a number of compounds containing 1,2,3-triazoles have shown a broad spectrum of biological activities such as antibiofilm inhibitors,[7] antibacterial,[8] antimicrobial,[9] and anticancer.[10] In general, the copper(I)-catalyzed 1,3-dipolar cycloaddition namely Huisgen–Fokin–Sharpless usually proceeds to completion in 6–36 h at ambient temperature in water with a variety of organic co-solvents, such as *tert*-butanol, ethanol, DMSO, THF, or $CH_3CN$, and this reaction is useful for large classes of azides and alkynes.[6] However, since the particular allylazides exist as an equilibrating mixture of regioisomers,[11] the reaction should be expected to be complex and might lead at least to mixtures of three 1,2,3-triazole species resulting from rearrangements of starting azides. In this context, we have first focused our interest on the feasibility of the one-pot reaction between geranyl bromide, propargyl methyl ether, and sodium azide, sodium ascorbate in DMF/$H_2O$ in the presence of $CuSO_4$–$H_2O$ as the catalyst. Formation of azide in dimethylformamide was first monitored by $^1H$ NMR (linalyl form 10%). The three-component reaction was then investigated following the process given in Scheme 1.

Under these conditions, two compounds were isolated and identified as 1-geranyl-4-methoxymethyl-1*H*-1,2,3-triazole **1a** and 1-neryl-4-methoxymethyl-1*H*-1,2,3-triazole **1b** in a 35/65 *Z*/*E* ratio. Assignment of the stereochemistry of **1a** and **1b** was unambiguously determined by $^{13}C$ NMR and by comparison with the parent compound geraniol and nerol: chemical shift of the methyl group attached to the isomeric double bond is of 17.8 ppm in the case of **1a**,[12] and of 23.5 ppm in the case of **1b**.[13] In this reaction, no formation of the 1-linalyl-1*H*-1,2,3-triazole derivative was found. However, since the yield of the reaction (66%) was not sufficient enough for our purpose, we decided to investigate a two-step procedure as follows (Scheme 2): azide was first prepared in refluxing acetonitrile, and was treated in order to eliminate excess of sodium azide and other salts (washed with brine and extracted with ethyl acetate). The resulting

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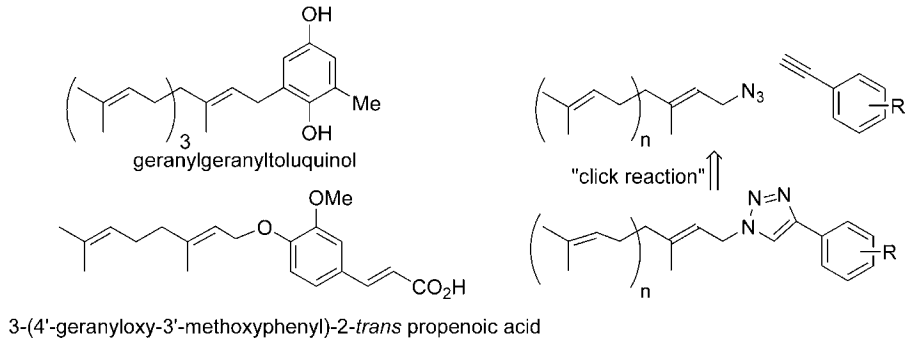
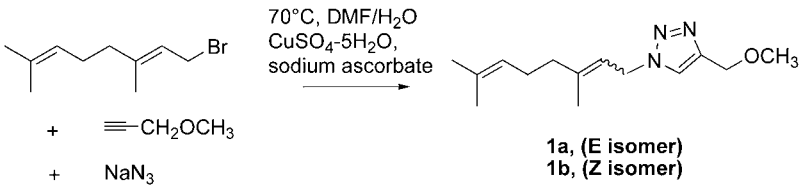
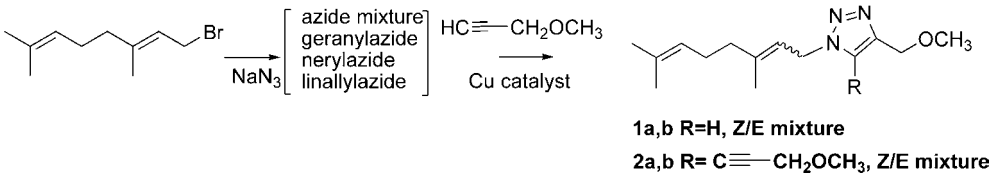



**Figure 1.** Structure of targeted library.




**Scheme 1.** Three-component synthesis of triazoles.




**Scheme 2.** Two-step procedure for the synthesis of triazoles.

non-purified mixture of the three allylic azides was monitored by $^1$H NMR and then submitted to a set of diverse conditions in order to optimize copper source, co-solvent, and temperature. Results are reported in Scheme 2 and Table 1.

The reported results clearly indicated the influence of the nature of catalyst. When using Cu wire, compounds **1a,b** were obtained admixed with the unexpected trisubstituted triazoles **2a,b** (entries 3 and 4). Such side reactions have been reviewed.[6] For example, Sharpless and co-workers have observed such derivatives in the direct synthesis of trisubstituted triazoles via addition of bromomagnesium acetylides to azides.[14] More recently, formation of such 5-alkynyl-1,2,3-triazoles from azides and alkynes was reported by Baudouin et al.[15] by use of tetrakisacetonitrile copper(I)hexafluorophosphate with a diamine ligand. However, in this study, the expected 1,4,5-trisubstituted triazoles were always obtained admixed with their 1,4-disubstituted derivatives. In this context, further optimization of reaction conditions indicated that using acetonitrile as co-solvent at 70 °C led exclusively to the 5-alkynyl-1,2,3-triazoles **2a,b** in 71% yield (entry 5). To our knowledge, this method using Cu wire was not described previously, and finally represent the simplest way to obtain 5-alkynyl-1,2,3-triazoles in good yields. Furthermore, we found that the synthesis of **1a**,**b** was better assumed using $CuSO_4$–$5H_2O$ with sodium ascorbate and dimethylformamide as co-solvent (entry 1).[16] In addition whatever the conditions used, no derivatives possessing the linalyl skeleton were found. Finally, conditions used in entry 1 were retained for designing a small library of terpenoid-like compounds by using geranylbromide and farnesylbromide as the source of terpenoid units and some ethynylmethoxybenzene derivatives as the source of terminal alkynes (Table 2).

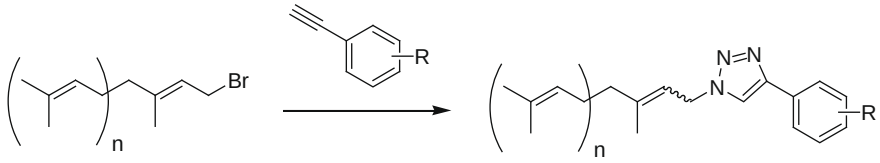
As in the case of geranylazide, farnesylazide underwent the allylic rearrangement (monitored by $^1$H NMR) without effect on

**Table 1**
Copper (I)-catalyzed synthesis of 1,2,3-triazoles 1 and 2 in various conditions[a]

| Entry | Cu source | Solvent/$H_2O$ (temp) | Compounds | |
|---|---|---|---|---|
| | | | **1a**,**b** (%, *Z*:*E* ratio) | **2a**,**b** (%, *Z*:*E* ratio) |
| 1 | $CuSO_4$/sodium ascorbate | DMF (rt) | 96% (32/68)[b] | 0% |
| 2 | $CuSO_4$/sodium ascorbate | $CH_3CN$ (rt) | 80% (48/52)[b] | 0% |
| 3 | Cu wire | DMF (rt) | 57% (50/50)[c] | 43% (50/50)[c] |
| 4 | Cu wire | $CH_3CN$ (rt) | 44% (40/60)[c] | 56% (40/60)[c] |
| 5 | Cu wire | $CH_3CN$ (70 °C) | 0% | 71% (31/69)[b] |

[a] All experiments were achieved in the same conditions of concentrations of reactants, catalyst, volume of solvents as referenced for entry 1 in Ref. [16].
[b] Calculated after purification.
[c] Calculated from $^1$H NMR spectra of crudes mixtures.

**Table 2**
Synthesis of selected 1,2,3-triazoles[a]




| Compounds **a**(*Z*),**b**(*E*) | *n* | R | Yield (%) | *Z*/*E* ratio |
|---|---|---|---|---|
| **3a,b** | 1 | 2-Methoxy | 83 | 20/80 |
| **4a,b** | 1 | 3-Methoxy | 92 | 36/64 |
| **5a,b** | 1 | 4-Methoxy | 68 | 38/62 |
| **6a,b** | 1 | 3,5-Dimethoxy | 45 | 41/59 |
| **7a,b** | 1 | 3-Hydroxy | 94 | 58/42 |
| **8a,b** | 2 | 2-Methoxy | 78 | 37/63 |
| **9a,b** | 2 | 3-Methoxy | 69 | 40/60 |
| **10a,b** | 2 | 4-Methoxy | 94 | 36/64 |
| **11a,b** | 2 | 3,5-Dimethoxy | 82 | 35/65 |
| **12a,b** | 2 | 3-Hydroxy | 88 | 35/65 |

[a] All experiments were achieved in the same conditions of concentration of reactants, catalyst, volume of solvents as referenced for entry 1 in Ref. [16].

the final composition. As observed (Table 2), independently of the structural differences within the azide precursors (geranyl or farnesyl), the 1,2,3-triazole derivatives were obtained as *Z*/*E* mixtures in good to excellent yields.

All compounds were tested as *Z*/*E* mixtures at a concentration of 500 μM for their capacity to inhibit biofilm formation by *Pseudoalteromonas* sp. D41. (Table 3).[17] Farnesol (the parent sesquiterpene of 8–12 and SEANINE® (a commercial biocide used in antifouling coatings) were used as references. The results showed that all farnesyl derivatives presented mild to good anti-adhesion activities, while only two geranyl derivatives were potentially active.

The most active compounds **5a**,**b** and **11a**,**b** were selected to evaluate the $EC_{50}$ of each isomer ($EC_{50}$ is expressed as the effective concentration to inhibit 50% of the bacterial adhesion). Results showed that both isomers of the farnesyl derivative are active. All compounds were also evaluated as the *Z*/*E* mixtures for their potential antibacterial activities, in order to correlate inhibition of biofilm to an antibacterial activity. Using the disk diffusion assay methods,[18] compounds were tested for their antibacterial activities against various strains of bacteria, including *Pseudoalteromonas* sp. (D41), *Pseudomonas aeruginosa* (ATCC A22), *Staphylococcus aureus* (ATCC 53.156), *Bacillus cereus* (ATCC 78.3), and *Escherichia coli* (ATCC 54.8 T). Interestingly, no significant inhibitory activities were observed when compared to norfloxacin.

In summary, a simple and efficient procedure allowing the rapid assembly of libraries of bioactive valuable 1,2,3-triazoles containing terpenoid mimetic structures has been developed. Furthermore, the potential of the Cu(I)-catalyzed Huisgen reaction as a successful strategy to explore new diversity space and address the structural decoration of privileged scaffolds has been established in this area. Although we did not obtain any antibacterial activity with the newly constructed terpenoid-like compounds, it can be concluded that this class of compounds provide interesting lead in our search for new potential inhibitors of bacterial biofilm formation that should be used as environmentally-friendly antifouling biocides. Further studies in this field by using others bacterial strains are actually under way.

**Table 3**
*Pseudoalteromonas* sp. D41 biofilm inhibition of compounds **1**–**12**

| Compounds[a] | (%) of adhesion[b] | $EC_{50}$ (μM) | | |
|---|---|---|---|---|
| | | *Z*/*E* (**a**,**b**) | *E* (**a**) | *Z* (**b**) |
| **5a,b** | 3 ± 8 | 400.0 ± 5.9 | 298.0 ± 1.2 | 188.0 ± 1.2 |
| **7a,b** | 30 ± 6 | | | |
| **8a,b** | 25 ± 7 | | | |
| **9a,b** | 15 ± 39 | | | |
| **10a,b** | 46 ± 7 | | | |
| **11a,b** | 0 ± 7 | 99.0 ± 1.2 | 71.0 ± 1.2 | 53.0 ± 1.2 |
| **12a,b** | 34 ± 2 | | | |
| **Farnesol** | 25 ± 9 | 188.0 ± 1.2 | | |
| **Seanine®** | 19 ± 6 | 135.0 ± 2.0 | | |

[a] No activity was observed with other compounds (**3, 4, 6**).
[b] Percentage of adhesion at a concentration of 500 μM (% of adhesion).

## Acknowledgment

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12. *1-Geranyl-4-methoxymethyl-1H-1,2,3-triazole* (**1a**): Yellow oil, MS (ESI), 250 (M+1), 272(M+23), $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.58 (s, 3H, $CH_3$), 1.66 (s, 3H, $CH_3$), 1.76 (s, 3H, $CH_3$), 2.12 (m, 4H), 3.39 (s, 3H, $OCH_3$), 4.55 (s, 2H), 4.94

(d, $J$ = 7.33, 2H), 5.04 (m, 1H), 5.41 (t, $J$ = 7.59, 1H), 7.47, (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 16.3, 17.6, 25.6, 26.0, 39.3, 47.7, 58.2, 66.0, 116.9, 121.5, 123.4, 132.1, 143.3, 145.0.

13. *1-Neryl-4-methoxymethyl-1H-1,2,3-triazole* (**1b**): Yellow oil, 250 (M+1), 272(M+23), $^1$H NMR: (400 MHz, $CDCl_3$) $\delta$:1.60 (s, 3H, $CH_3$), 1.67 (s, 3H, $CH_3$), 1.79 (s, 3H, $CH_3$), 2.17 (m, 4H), 3.39 (s, 3H, $OCH_3$), 4.56 (s, 2H), 4.92 (d, $J$ = 7.33, 2H), 5.08 (m, 1H), 5.41 (t, $J$ = 7.59, 1H), 7.49, (s,1H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 17.6, 23.3, 25.6, 26.2, 32.0, 47.6, 58.2, 66.0, 117.7, 121.6, 123.1, 132.6, 143.0, 145.0.
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16. Entry 1: A solution of geranyl bromide (0.78 mmol), and $NaN_3$ (2.8 mmol) in $CH_3CN$ (1.5 mL) was stirred at 70 °C for 2 h. A saturated solution of $Na_2CO_3$ was added, and the resulting solution was extracted three times with ethyl acetate. The organic layers were then dried over $Na_2SO_4$ and evaporated. The resulting oil was added to a solution of $H_2O$/DMF (1.5 mL/1.5 mL) containing $CuSO_4$–$5H_2O$ (0.07 mmol), propargyl methyl ether (1.1 mmol), and sodium ascorbate (0.2 mmol). The resulting mixture was stirred 12 hours at rt. A saturated solution of $Na_2CO_3$ was added, and the resulting solution was extracted three times with ethyl acetate. The organic layers were then dried over $Na_2SO_4$ and evaporated to give the corresponding triazoles, which were purified by flash chromatography on silica gel (Si60 15–40 μm, *n*-hexane/ethyl acetate (70:30)).
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