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Synthesis of jaspaquinol and effect on viability of normal and malignant bladder epithelial cell lines

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Abstract—The synthesis of jaspaquinol **1**, a monocyclic diterpene-benzenoid, is reported. Two synthetic routes to this natural product have been developed. The first, utilises a difunctional terpene derivative containing different leaving groups, facilitating the selective introduction of the cyclohexenyl and benzenoid fragments. The alternative route employs a regiospecific Stille cross-coupling reaction to introduce the cyclohexenyl fragment, which occurs without allylic transposition. Preliminary data shows the cell viability of **1** against normal and malignant human bladder epithelial cell lines.

1. Introduction

Jaspaquinol **1**,[1] isolated from two distinct marine sponges, *Suberea* sp. (order Verongida, family Aplysinellidae) and *Jaspis splendens* (order Choristida, family Jaspidae), has been reported by Crews and co-workers[2] and represents the first example of a monocyclic diterpene-benzenoid isolated from a natural source (Fig. 1).

It is the most potent human 15-lipooxygenase (15-HLO) inhibitor ($IC_{50} = 0.3\ \mu M$) isolated from their studies, and the proposed mode of action of **1** lies with reduction of an active Fe(III) enzyme to an inactive Fe(II) enzyme. It is highly plausible that the hydroquinol residue of **1** reduces iron,[2] however to date this has not been proven. The structure of **1** resembles rather closely farnesyl diphosphate (FDP) and geranylgeranyl diphosphate (GGDP), the former, which is an important substrate for squalene synthase (SQS)[3] and protein-farnesyl transferase (PFTase),[4] and the latter a substrate for protein geranylgeranyl transferase (PGTase, types I and II).[5] The recent synthesis of hydroxyaromatic FDP analogues by Jarman and co-workers[6] highlights this potential.

Following on from our own interest[7] in the development of new PFTase and PGGTase inhibitors, we herein report two synthetic routes to **1**. Retrosynthesis of **1** involves two disconnections at the C(7)–C(8) and C(15)–C(16) positions (Scheme 1).

Key to the synthesis of **1** is difunctional protected terpene intermediate **2**, which allows incorporation of the

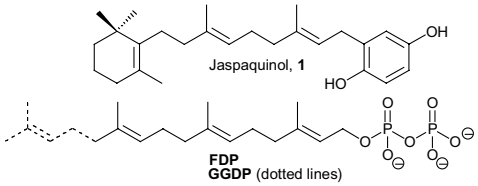
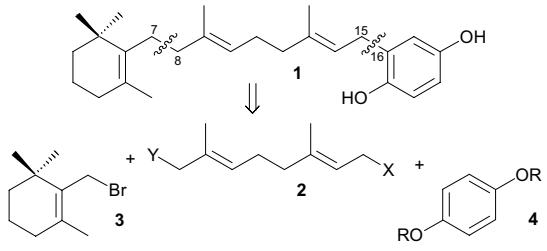


Figure 1.



Scheme 1. Retrosynthetic disconnection of jaspaquinol **1**.

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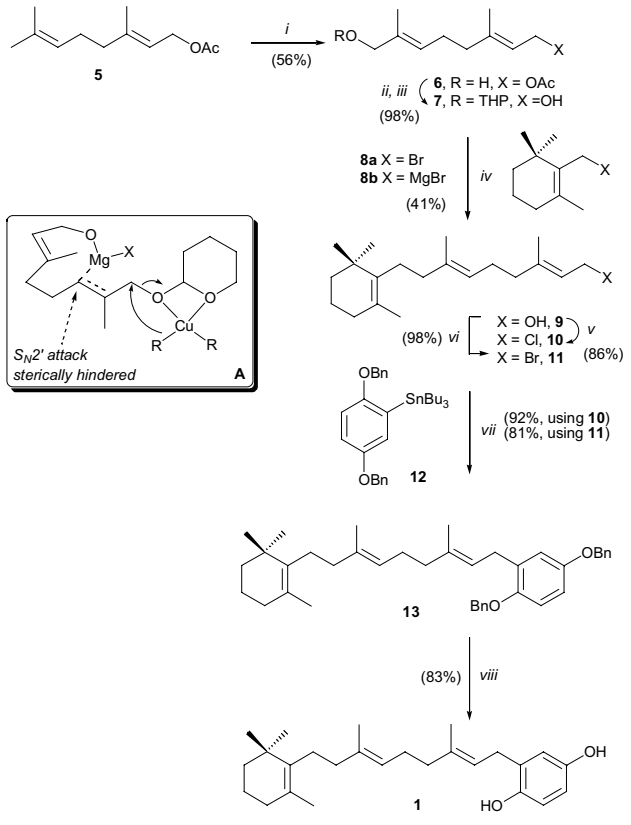
cyclohexene **3** and benzenoid **4** fragments by appropriate allylic substitution chemistry. The chosen protecting group strategy is crucial to the synthesis of **1** and has to be done in such a way, so as to avoid unwanted disubstitution and moreover proceed selectively.[8,9] If this challenge can be met, then there is great potential in the range of substituents/fragments that may be incorporated via transition metal mediated allylic substitution reactions.

The synthesis of derivatives, such as **2**, has received a vast amount of attention, particularly where X = OAc, OTBDMS, OBn, OTHP and Y = OH.[10] The *E*-selective allylic oxidation of the appropriately protected geranyl derivative using catalytic SeO_2 (5 mol%) and $^tBuO_2H$ (3.6 equiv) in CH_2Cl_2, developed in the early 1970's by Sharpless and Umbreit,[11] is still the method of choice (Scheme 2). However, there are some limitations when alternative protecting groups are employed that is, where X = OTBDMS, OBn, OTHP or OMe.[12] We chose the reactive acetate substrate **5**. Oxidation of **5**, under the conditions given above, followed by reduction of the crude material with $NaBH_4$, gave pure alcohol **6** in 56% yield. Alternatively, geranyl benzoate may be employed, which gives the oxidised product in similar yield (55%).



Scheme 2. Reagents and conditions: (i) SeO_2 (5 mol%), $^tBuO_2H$ (3.6 equiv), CH_2Cl_2, 25 °C, 8 h, then $NaBH_4$, EtOH, 0 °C, 1 h; (ii) DHP (1.2 equiv), TsOH (cat.), CH_2Cl_2, 25 °C, 12 h; (iii) K_2CO_3, MeOH, 0 °C, 6 h; (iv) EtMgBr (1 equiv), THF, 0 °C, then CuI, **8b** (formed by reaction with activated Mg in THF), THF, 50 °C, 6 h; (v) NCS, DMS, CH_2Cl_2, 0 °C, 2 h; (vi) NBS, DMS, CH_2Cl_2, 0 °C, 2 h; (vii) $Pd_2dba_3{\cdot}CHCl_3$ (2.5 mol%), PPh_3 (7.5 mol%), **12**, THF, 50 °C, 15–18 h; (viii) $LiAlH_4$ (3 equiv), $NiCl_2$ (1.1 equiv), THF, reflux, 11 h.

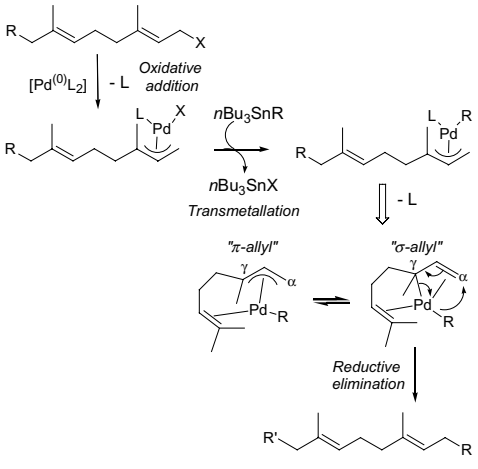
Protection of **6** as its tetrahydropyranyl ether (98% yield) and acetate hydrolysis using K_2CO_3 in MeOH gave key intermediate **7** in quantitative yield (55% overall yield from **5**).

The nucleophilic displacement of allylic tetrahydropyranyl ethers, such as **7**, with Grignard reagents (<5 equiv) under Cu(I) mediated conditions has been investigated in detail.[8] This method can be used here with slight modification. Initial reaction of the Grignard reagent with the alcoholic proton is key to the success of the selective S_N2 displacement, through carbomagnesiation of the magnesium alkoxide to the *distal* olefin, which blocks S_N2' attack. In order to keep the synthetic route efficient, we felt that reaction of **7** with ethyl magnesium bromide (1 equiv) followed by reaction with the Grignard reagent (2.1 equiv, **8b**) generated from known allylic bromide[13] **8a**, would give the required product selectivity. Under these conditions we were able to displace the tetrahydropyranyl moiety selectively in an S_N2 fashion to give the coupled product **9** in a modest 41% yield.

The chloride **10** and bromide **11** derivatives were prepared using a Corey et al. procedure.[14] So, **9** was reacted with *N*-bromosuccinimide/dimethyl sulfide and *N*-chlorosuccinimide/dimethyl sulfide in CH_2Cl_2, respectively, to give **10** and **11**, in good yield.

The Stille coupling of **10** and **11** was next investigated. Reaction of **10** with arylstannane **12**[15] in the presence of $Pd_2dba_3{\cdot}CHCl_3$ and PPh_3 gave the S_N2 product **13** in 92% yield, whereas use of the bromide **11** gave the S_N2 product in a lower 81% yield.

Coordination of the *distal* olefin is predicted to favour the S_N2 pathway via either π-allyl or σ-allyl intermediates (Scheme 3). The S_N2' pathway requires dissociation of the *distal* olefin[16] in order for the required *cis* insertion of the aryl species. The pathway is sterically hindered in both the dissociated and associated forms, although more so for the latter, and thus unlikely. Such selectivity has been observed for the addition of soft



Scheme 3. Selective S_N2 displacement.

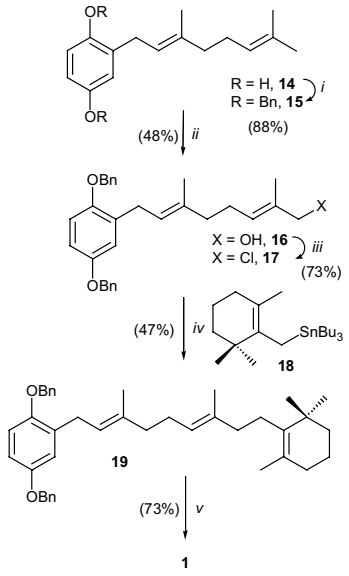
stabilised nucleophiles to geranyl derivatives under Pd or Mo catalysis.[17]

The final benzyl deprotection[15] was accomplished using $LiAlH_4$ in the presence of stoichiometric $NiCl_2$, which after 11 h gave jaspaquinol **1** in 83% yield. The structure of **1** was confirmed by comparison with the natural product (^1H and ^{13}C NMR), which is identical and provides verification of the reported structure.[1]

In the course of our synthetic investigations we further studied an alternative sequence to **1** (Scheme 4).

Compound **14** was prepared as reported.[18] Benzylation of **14** gave **15** in 88% yield. *E*-Selective oxidation of the terminal methyl group of **15** was accomplished using the $SeO_2/^tBuO_2H/CH_2Cl_2$ system to provide, after $NaBH_4$ reduction, alcohol **16** in 48% yield. The crude reaction mixture contained a number of polar byproducts, which were difficult to separate by flash chromatography.

Stille cross-coupling of **17**, available through reaction of **16** with NCS–DMS,[14] with allylic stannane **18** was envisaged as being an efficient way of introducing the cyclohexenyl fragment. Allylstannyl reagents generally react with allylic Pd-complexes by a different process to that normally observed for other organostannanes, in so much as allylic transposition in the tin reagent is sometimes seen. To this end, Godschalx and Stille[19] and Trost and Keinan[20] encountered allylic transposition in similar reactions. Notably, Schwartz discovered that maleic anhydride could be added to the reaction mixture to promote the formation of the desired 1,5-diene (acts as a π-acceptor ligand, which is proposed to accelerate reductive elimination).[21] We used similar reaction conditions to that reported, allowing the cross-coupled product **19** to be synthesised, after careful chromatography, in 47% yield. Several other products were isolated from this reaction, as a mixture, for which accurate characterisation was not possible. To complete the synthetic route, deprotection of the benzyl groups[15] was accomplished using identical conditions to that given in the first route, to give **1** in 73% yield.



Scheme 4. Reagents and conditions: (i) NaH (2.5 equiv), BnBr (3 equiv), DMF, rt, 24 h; (ii) SeO_2 (5 mol%), $^tBuO_2H$ (3.6 equiv), CH_2Cl_2, 25 °C, 8 h, then $NaBH_4$, EtOH, 0 °C, 1 h; (iii) NCS, DMS, CH_2Cl_2, 0 °C, 2 h; (iv) Pd(π-allyl)Cl]$_2$ (2 mol%), maleic anhydride (5 mol%), $RSnBu_3$ **18**, dry, oxygen-free THF, 60 °C, 15 h; (v) $LiAlH_4$ (3 equiv), $NiCl_2$ (1.1 equiv), THF, reflux, 12 h.

2. Human urothelial cell viability studies

The viability of normal human urothelial NHU cells treated with **1** at various concentrations (0–100 μM) for 24–72 h was determined by both phase contrast microscopy and MTT assay.[22] Cells exposed to doses of 0.78 μM and below remained morphologically normal, showed no effect on growth and entered exponential growth on day 3 (Fig. 2). Cells exposed to **1** at 100 μM showed a reduction in the number compared to controls by 24 h post-treatment ($p < 0.0001$) and were morphologically slightly rounded. By day 3, cells exposed to concentrations of 25 μM and above showed evidence of cell death, with cell debris visible (Fig. 3). In addition, a dose-dependent decrease in cell numbers compared to controls was observed at days 3 and 6 at concentrations of 3.125 M and above. These results suggest that **1** had no effect on cell growth and morphology before 24 h at concentrations below 100 μM, it then caused a reduction in cell growth at concentrations of 3.125 μM and above, and appeared to cause cell death at concentrations of 25 M and above.

Compound **1** was tested against a urothelial carcinoma EJ cell line, which contains the Harvey ras oncogene (Fig. 4). It was found that after 24 h, EJ cells were morphologically unaffected by treatment with **1**, with the exception that cell death was apparent at 100 μM concentration.

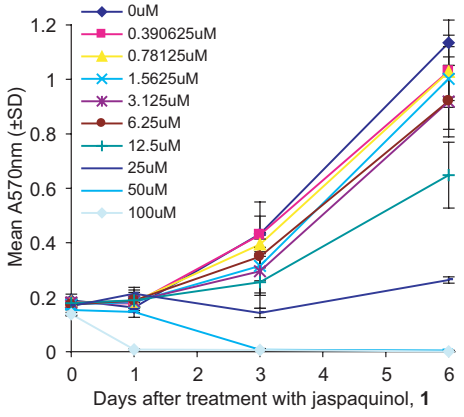


Figure 2. Effect of **1** on NHU cell growth. MTT assays were performed 0, 1, 3 and 6 days after the start of a 0–100 μM **1** treatment range applied to Y324 cells at P5. The mean absorbance at 570 nm (A570 nm) is proportional to the number of cells. Each data point is the average of six replicates, and standard deviations are shown by error bars.

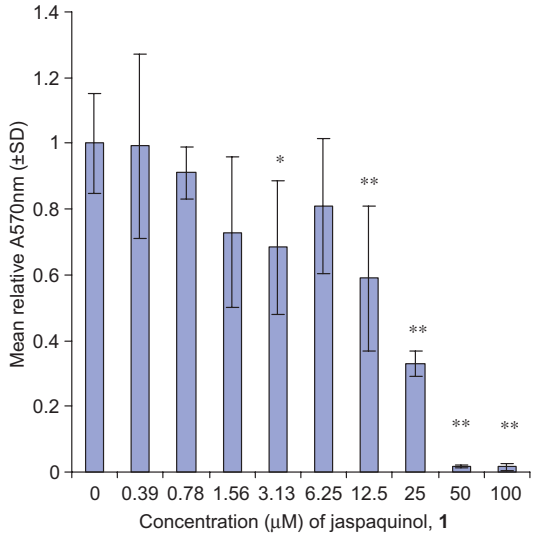


Figure 3. Effect of **1** on NHU cell growth at day 3. MTT assay at day 3. The mean relative A570 nm was determined by normalisation to a solvent control (0 μM treatment). Each data point is the average of six replicates, and standard deviations are shown by error bars. ($^{*}P < 0.05$, $^{**}P < 0.01$).

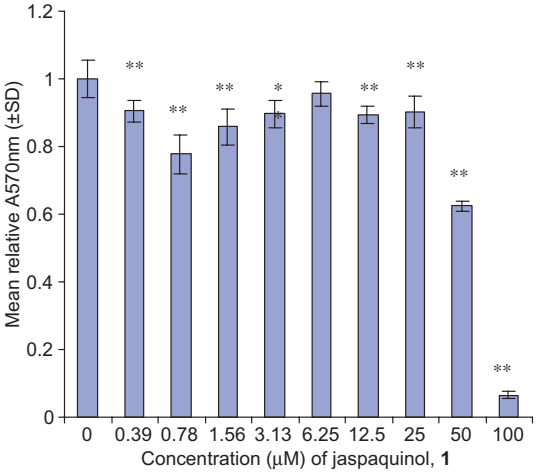


Figure 4. Effect of **1** on EJ cell growth at day 3. MTT assay at day 3. The mean relative A570 nm was determined by normalisation to a solvent control (0 μM treatment). Each data point is the average of six replicates, and standard deviations are shown by error bars. ($^{*}P < 0.05$, $^{**}P < 0.01$).

After 3 days, EJ cells showed no morphological differences in response to doses of 25 μM and under. Treatment with 50 μM of **1** caused some cell death, whilst there was increased cell death in response to 100 μM of **1**. The MTT data reflects these observations as there was a sharp drop-off in cell viability in response to doses over 25 μM.

In conclusion, we have identified two synthetic routes to jaspaquinol **1**. Each route has potential for the introduction of a range of hydrophobic and hydrophilic moieties. The viability of normal human urothelial NHU cells treated with **1** has been studied, which demonstrates that relatively high concentrations of **1** are required for cell death. Compound **1** was investigated for effects against a urothelial carcinoma EJ cell line, containing the Harvey ras oncogene. However, in this cell line at least, compound **1** does not inhibit the ras process. The full biological results, including the PFTase activity of **1** and related analogues, will be determined in due course.

Acknowledgements

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