Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3047–3052

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

CONFORMATIONALLY RESTRICTED PACLITAXEL ANALOGUES: MACROCYCLIC MIMICS OF THE "HYDROPHOBIC COLLAPSE" CONFORMATION

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Received 19 July 1999; accepted 14 September 1999

Abstract: Conformationally restricted macrocyclic analogues of paclitaxel were prepared, by connecting the 3'-phenyl group and the 2-benzoate moiety with two-atom tethers to mimic the "hydrophobic collapse" paclitaxel conformation. The analogues did not show activity in a tubulin assembly assay.

Paclitaxel (Taxol®), a complex natural product isolated from the bark of *Taxus brevifolia*, is an effective agent for the treatment of a variety of cancers.[3] We originally identified a predominant solution conformation for paclitaxel and docetaxel in which the 3'-phenyl, 2-*O*-benzoyl and 4-*O*-acetyl moieties are in close contact as determined by NOESY experiments in DMSO-d_6/D_2O.[4] Support for this conformation was subsequently reported for a water-soluble paclitaxel analogue in D_2O and in the crystal structure of paclitaxel.[5,6] This "hydrophobic collapse" conformation appears in all the active 3'-aryl analogues that we have subjected to rigorous conformational analysis.[7,8] Additionally, data from SAR studies show that deletion of any of the three moieties involved in the hydrophobic cluster results in decreased activity, while substitution of the 3'-phenyl or 2-*O*-benzoyl with sufficiently hydrophobic groups produces analogues which maintain excellent microtubule assembly activity.[9] These results led us to hypothesize that this conformation may be important for binding to microtubules.[10] In this communication we describe the synthesis of conformationally restricted macrocyclic paclitaxel analogues designed to mimic the hydrophobically collapsed conformation.

We selected the NOE contact between the *ortho* protons of the 3'-phenyl and 2-*O*-benzoyl moieties identified in previous NOESY experiments for initial design purposes. To preserve the aromatic-aromatic NOE contacts and the H2', H3' trans relationship[4] we selected linking the two rings through the *meta*-positions. Molecular modeling suggested that a two-atom *meta-meta* tether would best approximate the distance between the respective *ortho* positions in the hydrophobic collapse conformational model of paclitaxel and the *de novo* macrocyclic analogues. To this end, we chose to investigate the carbon-tethered olefin (**13**) and ethylene (**14**), and ester-tethered (**28**) analogues, shown in Schemes 2 and 4, respectively.

For the carbon-tethered analogues, we surmised that closure of the 18-member ring could be achieved by an intramolecular Heck reaction between an aromatic iodide and a terminal alkene. For our purpose, the aromatic iodide component of the Heck reaction could be derived from either the 3'-phenyl or 2-*O*-benzoyl rings. With no compelling reasons for either route, we selected and synthesized side-chain equivalent **5** (Scheme 1), which eventually places the aromatic iodide component in the 3'-phenyl group of the C13 paclitaxel side chain. The synthesis began with 3-iodobenzyl alcohol which was oxidized to aldehyde **1**, and subsequently transformed to the *N*-TMS imine **2** with lithium hexamethyldisilazide.[11] Asymmetric ester

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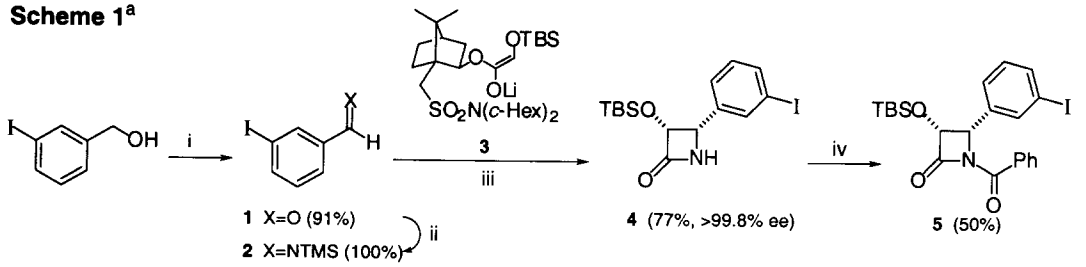
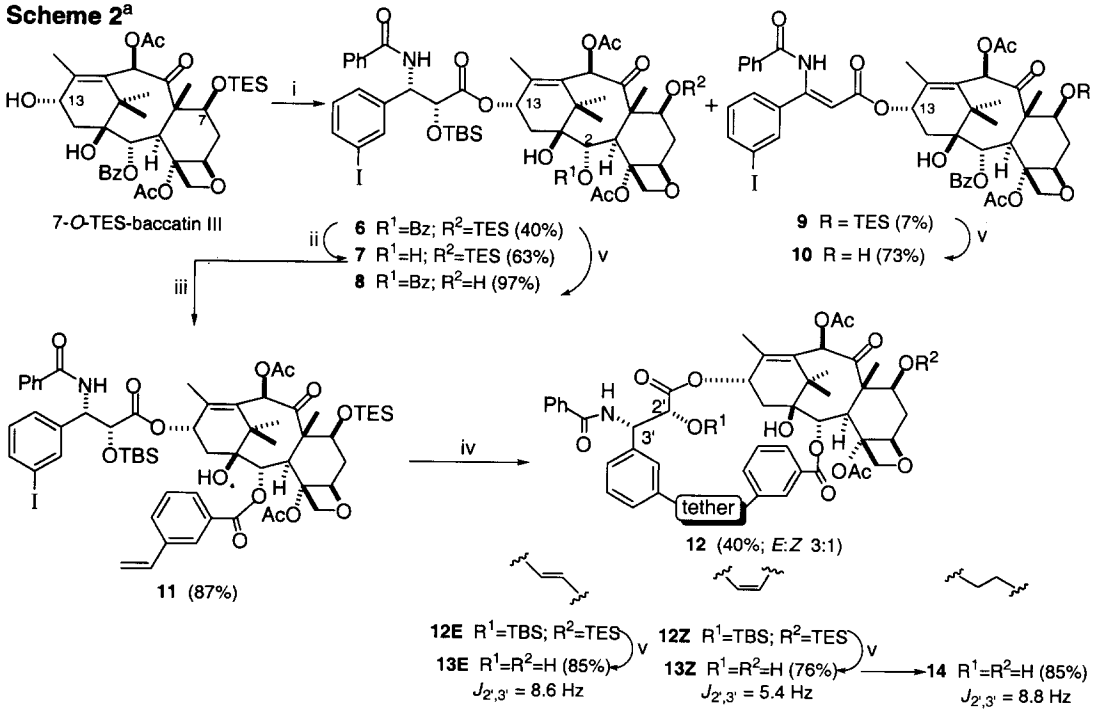
Scheme 1[a]



[a]i DMSO, Et_3N, CH_2Cl_2, -60 °C→ rt; ii LiHMDS, THF; iii THF, -78 °C→ rt; iv benzoyl chloride, Et_3N, DMAP (cat), CH_2Cl_2.

enolate-imine cyclocondensation of **2** and the enolate **3** gave 2-azetidinone **4** with 86% ee.[12] Recrystallization provided an essentially enantiopure compound (99.8% ee).[13] Acylation of the amide nitrogen with benzoyl chloride proceeded smoothly and quantitatively (by TLC), but isolation of the activated imide **5** proved difficult. We had noted this difficulty previously with an analogous 2-azetidinones possessing a *meta*-chloro substituent.[14] Coupling of the activated imide **5** and 7-*O*-TES-baccatin III resulted in the formation of **6** in 40% yield (Scheme 2). The low yield is apparently due to the presence of the *meta*-iodo substituting at the 4-phenyl group, as a low yield was observed before for coupling with a meta-chloro substituted acyl-azetidinone.[14] We suspect an instability, specific to this type of 2-azetidinone, may be contributing to the low

Scheme 2[a]



[a]i THF, NaH (10 equiv), **5** (10 equiv), 0→35 °C; ii *t*-BuOK (1.4 equiv), H_2O (1.2 equiv),THF, -42→-15 °C; iii 3-vinylbenzoic acid (20 equiv), DCC (20 equiv), DMAP (20 equiv), toluene, 55 °C; iv $Pd(OAc)_2$ (1 equiv), PPh_3 (3 equiv), $AgCO_3$ (2 equiv), CH_3CN, 60 °C; v pyridine-HF, pyridine; vi H_2, Pd/C, EtOAc.

yield of this reaction and the difficulty we noted isolating **5**. We also obtained **9** as a by-product (the geometry assigned by NOE), which could be attributed to base-catalyzed elimination of silanol from **6**.

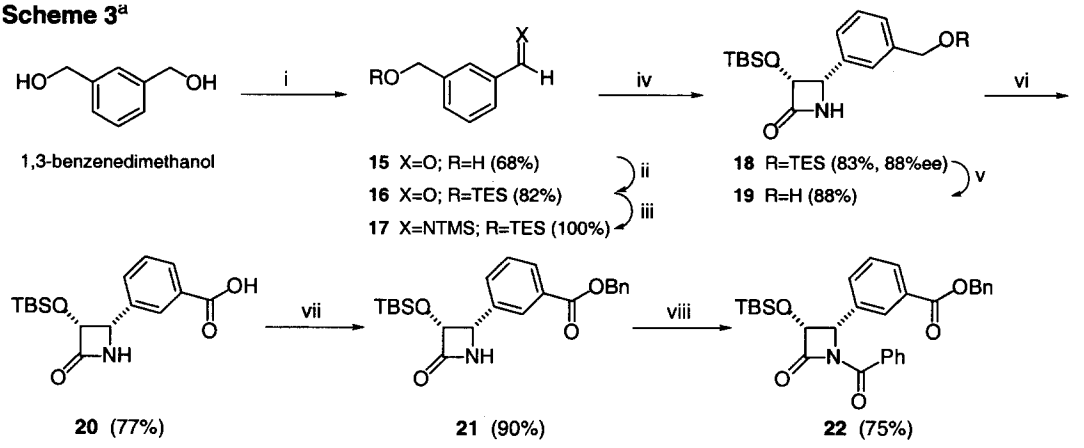
With the aromatic iodo component of the Heck macrocyclization installed, we turned to introduction of the alkene component (Scheme 2). The 2-*O*-debenzoyl intermediate **7** was prepared from **6** by selective hydrolysis of the O2-benzoate with anhydrous hydroxide, as we have reported earlier for the analogously protected paclitaxel.[15] Treating **7** with an excess of 3-vinylbenzoic acid, DCC and DMAP resulted in the macrocyclization precursor **11**. We investigated various conditions for the subsequent Heck reaction. Utilization of acetonitrile as solvent and $AgCO_3$ as an additive were critical for success. A stoichiometric amount of palladium "catalyst" was used to ensure an effective concentration of catalyst over the time course of the reaction. Two macrocyclic products (**12E** and **12Z**) were obtained from this reaction. The expected *trans*-macrocycle **12E** was predominant.

While TLC suggested a facile separation of the two macrocycles by silica chromatography, we were only able to isolate **12E** in pure form. Removal of the silyl protecting groups with pyridine-HF gave the stable, macrocyclic analogue **13E**. The macrocyclic intermediate **12Z** was consistently contaminated by its olefin isomer, suggesting a facile styrene-like isomerization process. Considering that our manipulations might be facilitating this process, we immediately deprotected this *Z*-macrocyclic enriched mixture with the hope that removal of the 2'-*O*-TBS group might stabilize the *Z*-geometry. To a certain extent this proved to be the case, in that we could now isolate the *Z*-macrocyclic analogue **13Z**. However, over time isomerization could be detected in solution (~12 h, $CDCl_3$) and in the solid-state (1:1 **13E-13Z** at one month) despite protection from light and air. The saturated macrocycle **14** was prepared by catalytic hydrogenation of an isomerized sample of **13Z**. The *E*-olefin (**13E**) and saturated (**14**) macrocyclic analogues exhibited the large H2'-H3' coupling constant typical of the hydrophobic collapse conformation.[4] The expected aromatic-aromatic and aromatic-4-acetyl NOE contacts and the characteristic large $J_{2'3'}$ coupling constant of this conformation were also noted for **13E**.[4] Insufficient material (and isomerization of **13Z**) precluded more detailed NMR experiments with **13Z** and compound **14**.

For the ester-tethered analogues, macrolactonization was envisioned as the key step (Scheme 4). Contrary to the analogues discussed above, the ester tether is not symmetric and the location of the carbonyl and alcohol components result in different compounds. Preliminary modeling suggested that the carbonyl component of the ester tether should come from the side-chain 3'-phenyl group for a close mimic of the hydrophobically collapsed conformation. The synthesis of side chain equivalent **22** began with 1,3-benzenedimethanol which was partially oxidized to aldehyde **15** (Scheme 3). Protected alcohol **16** was prepared from **15**, and subsequently transformed to the *N*-TMS imine **17**. The asymmetric ester enolate-imine cyclocondensation gave 2-azetidinone **18** with 88% ee from **3** and **17**. Selective hydrolysis of the TES ether resulted in benzyl alcohol **19**, which was oxidized to carboxylic acid **20**. The acid was converted to benzyl ester **21**, in order to provide an orthogonal deprotection at a later stage of the synthesis. Acylation of the amide nitrogen with benzoyl chloride provided activated imide **22**.

The ester macrocycles were prepared slightly differently because it seemed plausible that the anhydrous hydroxide conditions used to cleave the 2-benzoate (i.e., Scheme 2), could also hydrolyze the benzyl ester in the C13 side chain. Even though we will remove this protecting group later, we were concerned that

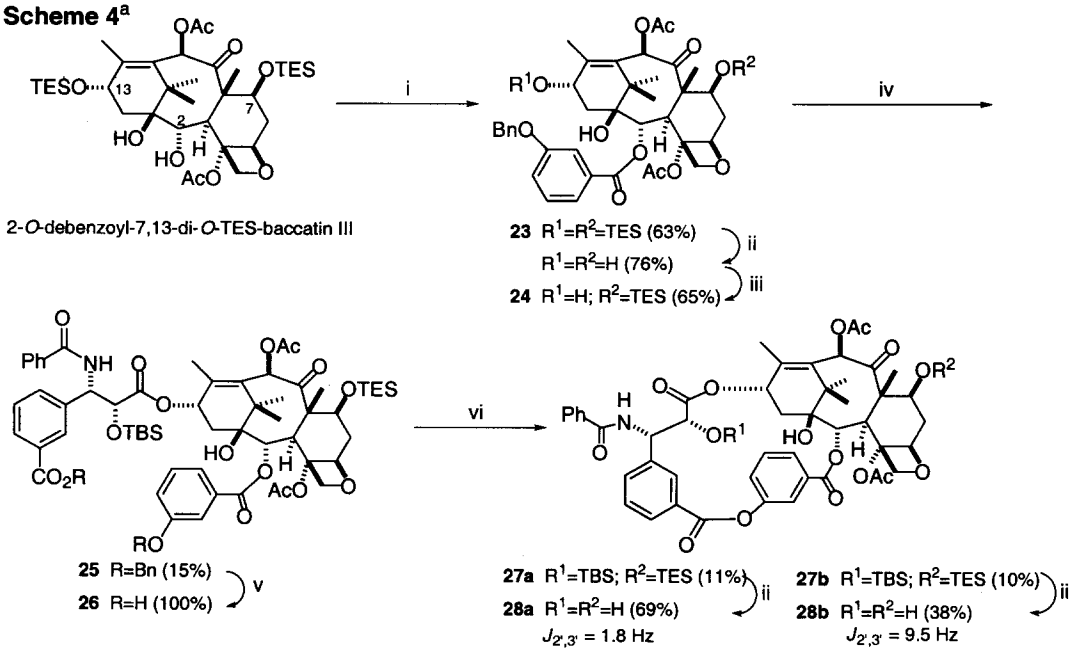
Scheme 3[a]



[a]i PCC, CH_2Cl_2; ii TESCl, Et_3N, CH_2Cl_2; iii LiHMDS, THF; iv **3**, THF, -78 °C→ rt; v 0.25% HCl/MeOH; vi 1) PDC, 2) $KMnO_4$; vii benzyl alcohol, DCC, DMAP, toluene; viii benzoyl chloride, Et_3N, CH_2Cl_2.

complications might arise during re-esterification of the 2-hydroxy group by either inter- or intramolecular self-condensation. Thus, 2-*O*-debenzoyl-7,13-di-*O*-TES baccatin III was transformed to **23** with excess 3-benzyloxybenzoic acid/DCC/DMAP (Scheme 4). Protecting group manipulation resulted modified baccatin III intermediate **24** ready for side-chain coupling with azetidinone **22**. As described above, the formation of **25**

Scheme 4[a]



[a]i 3-(BnO)benzoic acid, (20 equiv), DCC (20 equiv), DMAP (20 equiv), toluene, 55 °C; ii pyridine-HF, pyridine; iii TESCl, pyridine; iv **15**, NaHMDS, THF, -78 °C→rt; v H_2, Pd/C, THF; vi BOP-Cl, Et_3N, CH_2Cl_2.

was uncharacteristically poor (15%), and the 13-*O*-benzoyl-baccatin III (6%) byproduct was noted. Catalytic hydrogenolysis of the benzyl protecting groups resulted in the macrolactonization precursor **26**. Macrolactonization of **26** was mediated by BOP-Cl under high-dilution conditions. Interestingly, two products **27a** and **27b**, with identical mass (corresponding to the expected product), were isolated from this reaction.

Considering the potential isomeric products, a 12-member macrocycle (due to intramolecular esterification of O1) and 17-member macrocycle (due to O2-to-O1 acyl migration) could be ruled out by ^{13}C NMR (no downfield shift of C1 compared to paclitaxel). In fact, NMR spectra of these products were unremarkable except for the H2'-H3' coupling constant which was 1.8 Hz for **27a** and 7.8 Hz for **27b**. We suspect that these products are atropisomers. Indeed, more detailed modeling of the expected macrolactone, suggests at least four local minima of similar energy. Deprotection resulted in the macrolactone analogues **28a** and **28b**. Interestingly, NMR analysis of **28a** with the small H2'-H3' coupling constant shows all of the aromatic-aromatic NOE contacts expected for the hydrophobic collapse conformation, while **28b** possessing the large coupling constant does not, indicating distortion of the side chain from the desired conformation despite the expected value of $J_{2',3'}$. Also, **28b** appears to undergo a spontaneous conversion to an unidentified atropisomer with a small side-chain coupling constant in $CDCl_3$ solution over time.

The macrocyclic analogues **13**, **14**, and **28** were evaluated by the microtubule assembly method.[9] Because these analogues possess the potential to isomerize (especially **13Z** and **28b**), the assay was performed immediately following isolation. The analogues possessing the ester tether were inactive.[16] The hydrophobic collapse mimics **13E** and **14** (based on large $J_{2'-3'}$ only), of the carbon tethered series also exhibited no significant microtubule assembly. Analogue **13Z** which does not appear to adopt the hydrophobic collapse conformation (based on small $J_{2'-3'}$ only) also exhibited no activity. We also took the opportunity to examine the biologic activity of two new taxanes **8** and **10**, which were prepared as described in Scheme 2. The microtubule assembly shown by **8** is in accordance with previous 3'-phenyl SAR. Taxane **8** was less active than paclitaxel (microtubule assembly assay: $ED_{50}/ED_{50paclitaxel} = 4.6$ and cytotoxicity against B16 melanoma cells: $ED_{50}/ED_{50paclitaxel} = 27$).[16] Compound **10** was inactive in these assays.

One could speculate that the paclitaxel binding site recognizes the hydrophobic collapse conformation presented by these analogues. But because these analogues are tethered, a mutual ligand-protein conformational change that ultimately results in stabilization cannot occur. If this is the case, **13E** should compete with paclitaxel for the binding site resulting in inhibition of assembly. However, a concentration of 20 μM **13E** did not inhibit the assembly of tubulin stimulated by 1 μM paclitaxel, suggesting this macrocyclic analogue does not act as a "paclitaxel antagonist."

Since the macrocyclic compounds did not show microtubule stabilization and were unable to inhibit paclitaxel binding (**13E**), we believe that this may be indirect evidence that the bound state is unlike the "hydrophobic collapse;" however, a similar conformation has been recently invoked as a basis for designing a paclitaxel/epothilone hybrid, which has one third of the activity of paclitaxel.[17] The best published evidence to date is an electron diffraction, although the bound taxane was not well resolved and the structure depicted is docetaxel.[18] More recent work has led to the conclusion that the bound state is in fact highly extended, with the only remaining taxane intramolecular hydrophobic contact involving the 3'-phenyl and the 4-acetyl groups.[19] We believe that our results reported here are consistent with this model.

Acknowledgments: These studies were supported by The National Cancer Institute. We would like to thank the Scientific Education Partnership of Hoechst Marion Roussel for postdoctoral fellowship support to TCB. The authors would also like to thank Drs. G. Grunewald, C.-H. Czaplinski, R. K. Jalluri and S. Victory for their help with molecular modeling.

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