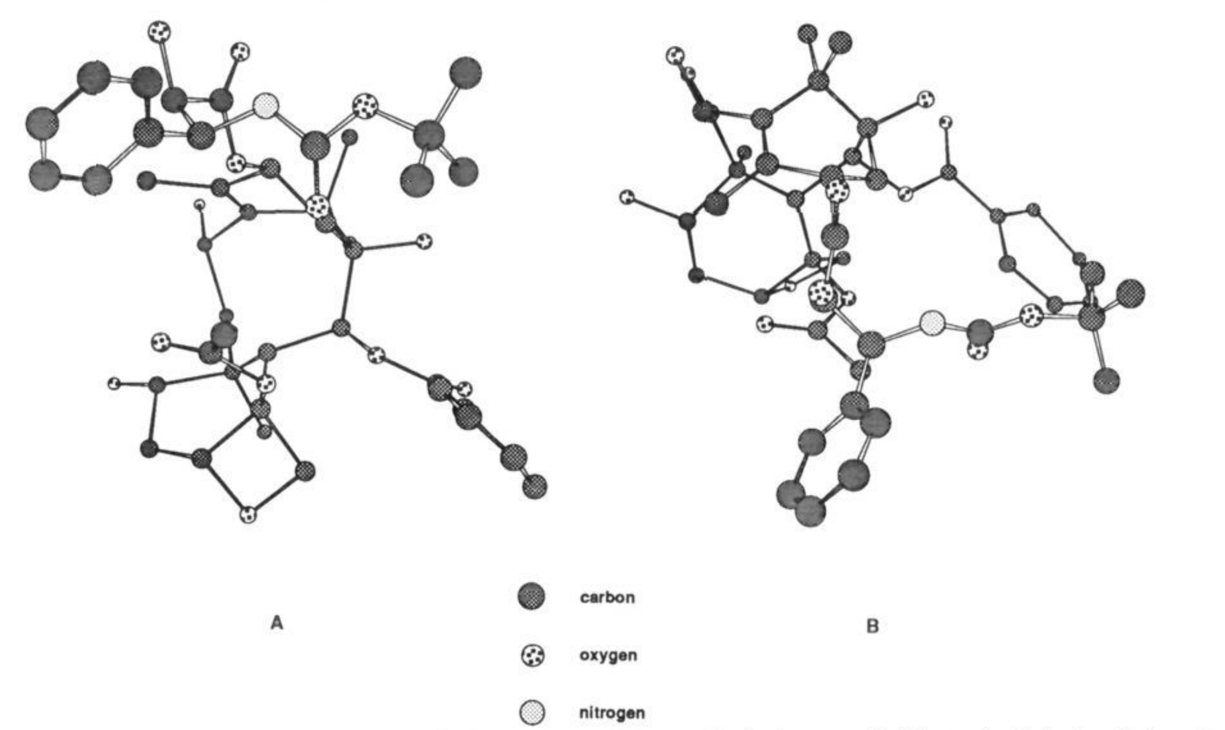

Figure 2. Crystal structure of taxotere (17) viewed with the A-ring side chain in the foreground. Orientation B is viewed along the side chain carboxyl carbon–oxygen single bond with the oxygen–C-13 bond in a vertical plane perpendicular to the page. Hydrogen atoms are deleted. Fractional coordinates are taken from ref 23.

Table IV. Solubility of Taxol and **3**–**8**[a]

drug	H_2O, μM	$CHCl_3/H_2O$	octanol/H_2O
taxol	35	>1000	>1000
3	820	160	33
4	1000	170	35
5	175	340	800
6	160	320	800
7	615	200	56
8	565	230	84

[a] All measurements were performed by reverse-phase HPLC (Merck-Hitachi instrument; methanol–water, 65:35).

bulin antibodies. At the ED_{50} concentrations of both taxol and **5**–**7**, there was no significant different between the ability of taxol and the analogues to induce the formation of microtubule bundles in CHO cells (data not shown).

Water Solubility of Taxol and Analogues. One of the major drawbacks of taxol as a prospective antitumor drug is its low water solubility that complicates its formulation. We examined the water solubility of analogues **3**–**8** by three different methods. In the first, an excess of drug was mixed vigorously with and sonicated in distilled water. The suspension was centrifuged and the clear supernatant analyzed for drug concentration. In the second and third, each compound was distributed between a two-phase mixture of chloroform–water and octanol–water, respectively. The samples were mixed vigorously for 1 h, and after separation of the two phases, each phase was examined by HPLC. The evaluation of drug concentration by HPLC is superior to determination by measurement of UV absorbance as it eliminates interference by products of drug degradation (i.e. hydrolysis). Table IV summarizes the results. All of the analogues have better water solubility than does taxol. Within the set of analogues investigated, hydrophilicity ranges, as might be expected, from the most soluble lactates (**3** and **4**) that lack a 3′-substituent, through the moderately soluble *N*-benzoylisoserinates (**7** and **8**) that have a modestly polar benzamido group at C-3′, to the least soluble phenyllactates (**5** and **6**) with the hydrophobic phenyl positioned at the termini of their side chains.

Discussion

Although there is a range of tubulin polymerization activities associated with taxol analogues **3**–**8**, it is encouraging from the point of view of analogue design that the rather profound structural alterations encompassed by this group still support reasonable activity. Nevertheless, it is obvious that certain features of the taxol side chain enhance activity. In particular, the presence of a hydrophobic residue at the terminus, as in phenyllactates **5** and **6**, is apparently advantageous. This is somewhat unfortunate given the need for a taxol analogue with better formulation properties[5] and the poor aqueous solubility associated with the presence of the taxol side chain. According to our measurements, the maximum water solubility of baccatin III is 1.44 mM, which is ca. 40 times higher than the solubility of taxol (see Table IV).

The most impressive aspect of the structure–activity function for **3**–**8**, however, is related to the side chain C-2′ configuration. Surprisingly, there is little dependence of activity on stereochemistry at that site in the lactate and phenyllactate analogues. Indeed, the unnatural *S* configuration in each diastereomeric pair appears to support slightly better activity. In contrast, when the amide substituent is retained in the side chain, significant dependence on C-2′ configuration is turned on such that the natural *R* configuration is preferred. Cast in terms of affinities for the binding site on microtubules, the two phenyllactate analogues would be equally avid ligands while the two *N*-benzoylisoserine analogues would not. Although the presence of the 3′-amide substituent is not obligatory for activity, as the results with **5** and **6** attest, it seems likely that when it does form a part of the side chain, the way in which it organizes that structure is important.

To gain some insight into this point, it is useful to consider the recently reported crystal structure of the taxol

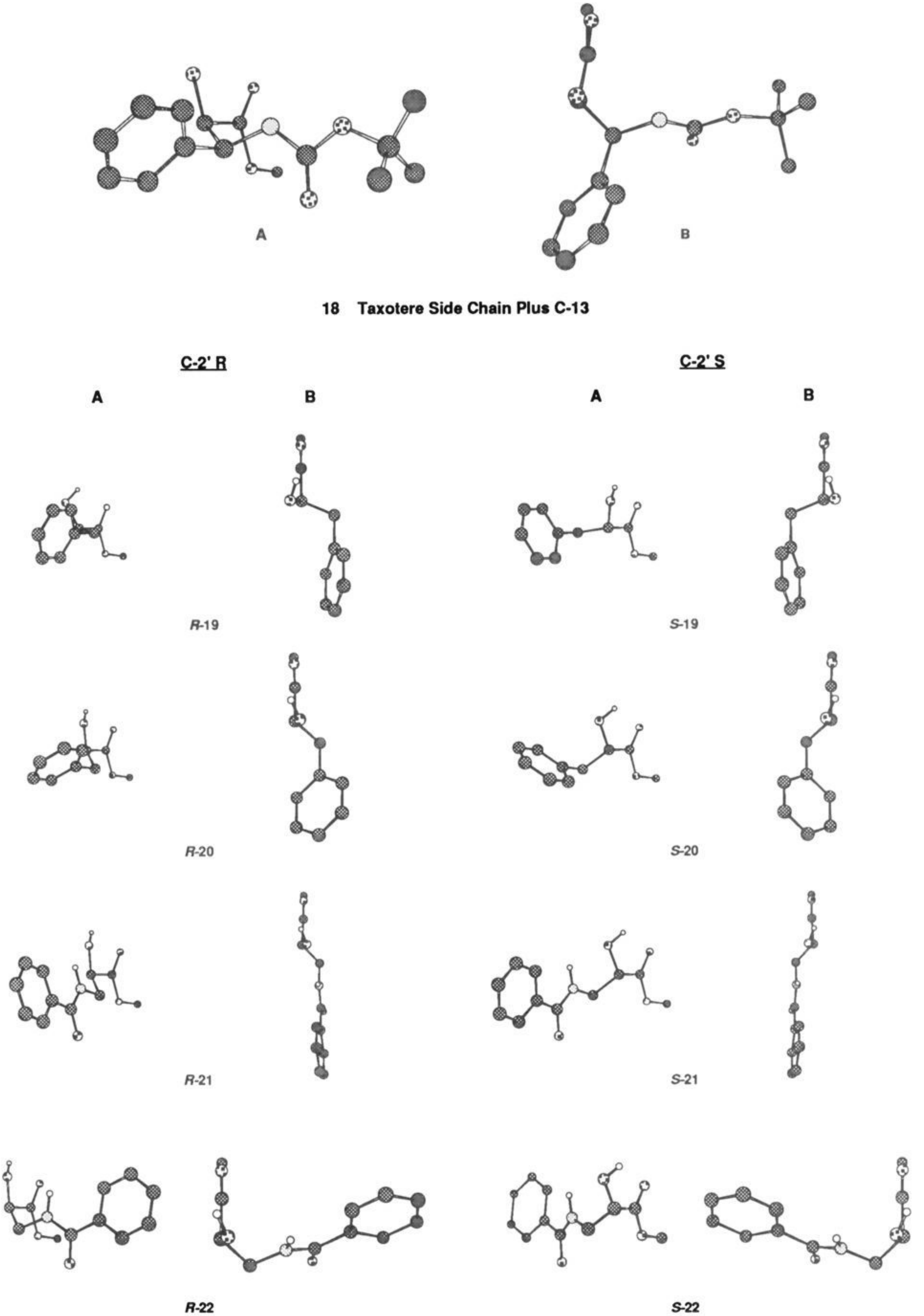

Figure 3. The taxotere side chain excised from the crystal structure and viewed as in A and B, Figure 2, and the optimized conformations of the side-chain methyl esters viewed similarly. Models **19** and **20** are of the methyl phenyllactates and models **21** and **22** are of the methyl *N*-benzoylisoserinates. Most hydrogen atoms are deleted for clarity.