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The *tert*-butyl dimethyl silyl group as an enhancer of drug cytotoxicity against human tumor cells

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Abstract—In this study, we synthesized a series of enantiomerically pure (2*R*,3*S*)-disubstituted tetrahydropyranes with diverse functional groups using known methodologies. In addition to the *tert*-butyl dimethyl silyl group, other common protecting groups for hydroxyl groups such as allyl, acetate, and benzoate were used to obtain appropriate derivatives. Pure compounds were evaluated in vitro against HL60 human leukemia cells and MCF7 human breast cancer cells. From the growth inhibition data a structure–activity relationship was obtained. Overall the results point to the relevant role of the *tert*-butyl dimethyl silyl group in the modulation of cytotoxic activity.

Cytotoxic drugs continue to play a major role in cancer therapy. Some cytotoxic agents have been improved via pro-drug approaches. Improvements have been highly drug and disease specific, and suffer from drawbacks with respect to the efficiency of cellular uptake and drug release. Among the strategies proposed to enhance the passive internalization of drugs into cells, increasing their lipophilicity has often been demonstrated as a successful way. For instance, fatty acid derivatives of ara-C[1] and gemcitabine[2] showed better retention profile and antitumor effect in leukemia, solid tumor cell lines, and human xenografts. Similarly, lipophilic platinum(II) complexes were reported to be more active against tumor cell lines,[3] and the activity could be correlated to the drug lipophilicity.[4]

An interesting approach that has not been exploited sufficiently to increase drug lipophilicity is the use of silicon. Besides the bond length, the difference between carbon and silicon that may be relevant in drug design is the increased lipophilic character of silicon, which may modulate drug cytotoxicity via increasing cellular uptake. Note that the hydrophobic fragment constants for C and Si are 0.20 and 0.65, respectively.

It is exceptional to find examples of silicon containing anticancer drugs in the literature.[5] However, cisplatin[6] and camptothecin[7] analogs containing silicon have been reported to give a better activity profile than their respective parental drugs. To investigate the cytotoxic modulation of silicon, we prepared and tested two series of enantiomerically pure (2*R*,3*S*)-disubstituted tetrahydropyranes containing diverse functional groups. These heterocycles are common intermediates in our stereoselective synthesis of cyclic ethers and were selected as model for our study.[8]

The rationale behind our strategy is to introduce lipophilicity by the addition of another moiety, the *tert*-butyl dimethyl silyl (TBS) group. This silicon scaffold was selected because it is widely used in synthetic organic chemistry as a protecting group of hydroxyl groups. In

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addition to the TBS group, other common protecting groups for hydroxyl groups, such as allyl, acetate, and benzoate, were used to obtain appropriate derivatives. We report herein the synthesis and preliminary in vitro inhibitory activity against HL60 human promyelocytic leukemia cells and MCF7 human breast cancer cells. These cell lines are included in the National Cancer Institute anticancer screening program and are widely found in literature as representative examples of leukemias and solid tumors, respectively.

The synthesis of the compounds used in this study is outlined in Scheme 1. Tetrahydropyran derivatives **2**–**17** were envisioned as being synthesized from the common precursor alcohol **1**,[9] which can be obtained from the commercially available tri-*O*-acetyl-D-glucal. A first series of compounds was prepared from nitrile **2**. To carry out the synthesis of the nitrile compound **2**, a one-carbon homologation of the alcohol **1** was performed by a simple two-step sequence; tosylation and NaCN nucleophilic substitution. When compound **2** was submitted to reduction with DIBAL-H in toluene at low temperature tetrahydropyran **3** was obtained as a side product and in low yield. On the contrary, the reduction of **2** with DIBAL-H in THF at 0 °C led to aldehyde **4** in 92% yield. Aldehyde **5** was obtained from nitrile **2** by a three-step process; deprotection of the silyl ether, O-alkylation with ally bromide, and reduction with DIBAL-H. The two-carbon homologation of **4** by a Wittig–Horner reaction afforded the (*E*)-α,β-unsaturated ester **6**. The TBS of the latter compound was cleaved to give the secondary free alcohol **7**. The DMAP catalyzed acetylation and benzoylation of **7** led, in high yields, to the acetyl and benzoyl esters **8** and **9**, respectively. Reduction of **6** with in situ generated alane afforded the allylic alcohol **10**.

A second series of compounds having one carbon atom less than their homologues of the previous series was prepared. Thus, aldehyde **11** was obtained by Swern oxidation of the alcohol **1** in 86% yield. Subsequent Wittig–Horner reaction led to the (*E*)-α,β-unsaturated ester **12**. This ester was reduced with alane and the allylic alcohol **13** was obtained. Acid catalyzed deprotection of the TBS ether gave the diol **14**. Finally, the selective protection of the primary alcohol as a TBS ether yielded the alcohol **15**. In addition, (*Z*)-α,β-unsaturated ester **16** was prepared by a Wittig–Horner reaction of aldehyde **11** using the Ando reagent.[10] Further reduction with DIBAL-H in THF at 0 °C provided the (*Z*) allylic alcohol **17** in good yields.[11]

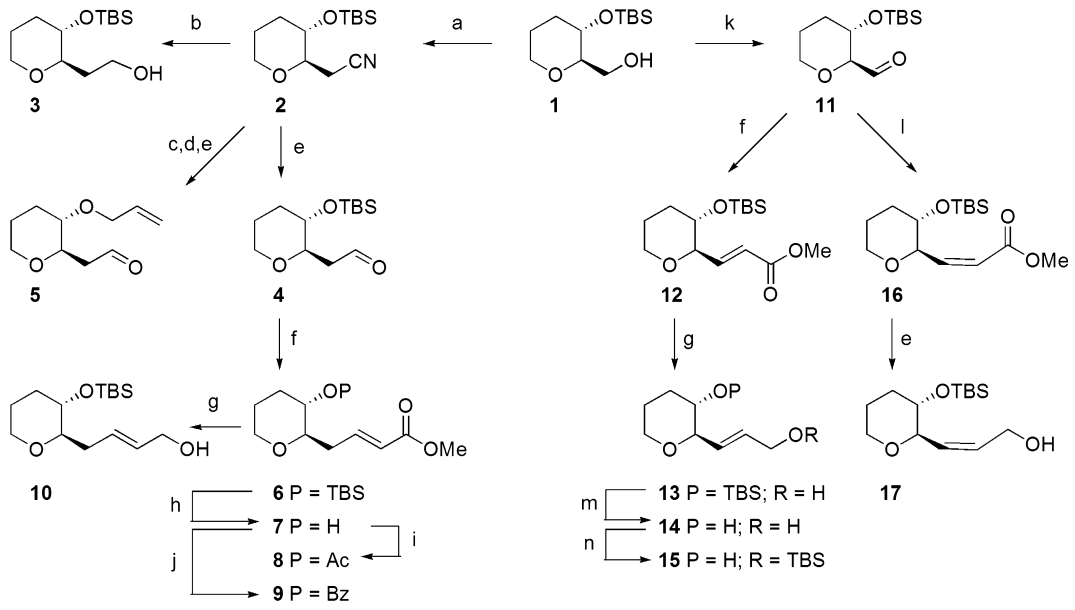
Cell antiproliferative assays were performed in 96-well plates using the National Cancer Institute protocol with slight modifications.[12] We screened growth inhibition and cytotoxicity against HL60 and MCF7 cells after 48 h of stimulation using the sulforhodamine B (SRB) assay.[13] Additionally, treated and control cells were analyzed by phase contrast microscopy to assess morphological changes. Three dose–response parameters can be calculated, when possible, for each experimental agent from the dose–response curves. Growth inhibition of 50% (GI_{50}), which is the drug concentration resulting in a 50% reduction of cellular net growth when compared with values of untreated control cells, the drug concentration resulting in total growth inhibition (TGI), and the net loss in 50% of cells following treatment (LC_{50}) denoting cell kill.

The octanol/water partition coefficient expressed in logarithmic form (CLOGP) has been widely used in calculating numerous physical properties such as membrane transport and water solubility. It is usually calculated from the sum of partition coefficients of the chemical fragments composing the molecule. The lipophilicity of the compounds was evaluated by in silico calculation based on their chemical structure.[14,15] CLOGP values were calculated to correlate lipophilicity with antitumor activity.

The CLOGP values together with the growth inhibition data are listed in Table 1. Taken as a whole, those compounds having a TBS group show larger CLOGP values and are therefore more lipophilic than the corresponding derivatives without TBS. The growth inhibition results allow us to classify the compounds according to their activity profile. The group of active compounds against HL60 cells comprises seven products, whilst MCF7 cells were sensitive to nine products. Overall, the active products showed GI_{50} values in the range 22–46 and 6.4–86 μM against HL60 and MCF7 cells, respectively. The breast cancer cell line was more sensitive to the drugs than the leukemia cells. For instance, compounds **15** and **16** were active against MCF7 cells but showed no activity against HL60 cells. Compounds **6**, **12**, and **17** were the only products from the active group series that reached a TGI value in HL60 cells. The TGI values for those products were in the range 64–77 μM. For MCF7 cells, compounds **4**, **6**, **10**, **12**, **16**, and **17** showed TGI values in the range 23–83 μM. Interestingly, compound **17** was the only product that reached a LC_{50} value in both cell lines and therefore, it appears as the most active compound of the series.

When the obtained dose–response parameters are considered, the following structure–activity relationship is obtained. In general, those compounds bearing a TBS protecting group at position 3 of the tetrahydropyran ring showed significant cytotoxicity in all cell lines whereas the compounds lacking such functionality were found inactive. Thus, when the TBS group is replaced with an allyl group (**4** vs **5**) the activity is lost. A similar observation is found when the TBS group is removed (**6** vs **7**, and **13** vs **14**) or substituted by an acetyl (**6** vs **8**) or a benzoyl group (**6** vs **9**).

The presence of a TBS group is not the unique requirement for the compounds to induce activity. Inactive compound **2** led us to consider the substituent at position 2 of the tetrahydropyran ring responsible for the activity exerted by the compounds. This can be seen also for compounds **13–15**. The removal of the TBS group of active product **13** led to inactive diol **14**. When the primary hydroxyl group was protected with TBS, the resulting derivative **15** was inactive in HL60 cells and showed slight activity in MCF7 cells.



Scheme 1. Reagents and conditions: (a) i—TsCl, Py, DMAP, room temperature (rt); ii—NaCN, DMSO, 80 °C, 66%; (b) DIBAL-H, toluene, −78 °C, 14%; (c) Bu_4NF, THF, rt, 64%; (d) allyl bromide, NaH, Bu_4NI, THF, 0 °C to rt, 89%; (e) DIBAL-H, THF, 0 °C, for **4** 92%, for **5** 67%, for **17** 76%; (f) $(MeO)_2POCH_2CO_2Me$, C_6H_6, NaH, rt, for **6** 78%, for **12** 88%; (g) $LiAlH_4/AlCl_3$, Et_2O, −20 °C, for **10** 93%, for **13** 70%; (h) Bu_4NF, HF, rt, 52%; (i) Ac_2O, DMAP, CH_2Cl_2, 86%; (j) C_6H_5COCl, DMAP, CH_2Cl_2, 80%; (k) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C, 86%; (l) $(PhO)_2POCH_2CO_2Me$, THF; NaH, −78 °C, 86%; (m) DOWEX 50W×8, MeOH, 68%; (n) TBSCl, imidazole, CH_2Cl_2, rt, 75%.

Table 1. In vitro cytotoxic activity of (2*R*,3*S*)-disubstituted tetrahydropyranes against HL60 leukemia cells and MCF7 breast cancer cells[a]

Compound	CLOGP[b]	HL60			MCF7		
		GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
2	2.626	na			na		
3	2.599	46 (± 22.3)			55 (± 17.6)		
4	2.757	40 (± 8.0)			21 (± 11.0)	82 (± 16.3)	
5	0.767	na			na		
6	3.651	22 (± 6.9)	77 (± 24.0)		18 (± 2.7)	48 (± 13.5)	
7	0.270	na			na		
8	1.172	na			na		
9	2.896	na			na		
10	2.865	27 (± 4.8)			25 (± 4.1)	83 (± 29.3)	
12	3.517	22 (± 14.0)	69 (± 43.8)		24 (± 8.3)	80 (± 35.1)	94 (± 10.1)
13	2.535	31 (± 15.6)			61 (± 15.8)		
14	−0.846	na			na		
15	2.499	na			86 (± 20.2)		
16	3.517	na			32 (± 14.3)	82 (± 26.0)	
17	2.535	26 (± 8.9)	64 (± 29.9)	88 (± 20.2)	6.4 (± 7.7)	23 (± 11.5)	63 (± 19.3)

[a] Values are given in μM and are means of two to four experiments, standard deviation is given in parentheses (na = not active). TGI and LC_{50} values are given only if they are less than 100 μM, which is the maximum concentration test.
[b] Ref. 14.

Compounds **2–10** have a longer side chain (one methylene group more) when compared to the derivatives **12–17**. A slight difference in activity is observed in favor of compounds with a larger side chain. Additionally, we prepared compounds that differ in the stereochemistry of the double bond; *E* for **12–15** and *Z* for **16–17**. Unlike the chain length, the stereochemistry of the double bond seems to play an important role in activity. Thus, the (*Z*) allylic alcohols are more potent than (*E*) analogs (**17** vs **13**), while (*E*)-α,β-unsaturated esters are more active than the corresponding (*Z*) products (**12** vs **16**).

In summary, we have constructed a series of (2*R*,3*S*)-disubstituted tetrahydropyranes in a simple and direct way. Although the results are preliminary, we found that these synthetic derivatives bearing a TBS protecting group at position 3 of the ring considerably induced cytotoxicity in HL60 human leukemia cells and MCF7 breast cancer cells in vitro. On the basis of growth inhibition parameters, a structure–activity relationship was obtained. Overall, the results show that TBS may not be seen only as a protecting group for organic synthesis, but as a plausible strategy to introduce lipophilicity in drugs which is anticipated to aid the cellular uptake.

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13. Pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration, that is, 100 μM. Control cells were exposed to an equivalent concentration of DMSO. Each agent was tested in duplicate at five different 10-fold dilutions. Drug incubation times were 48 h, after which cells were precipitated with 50 μL ice-cold 80% (w/v) trichloroacetic acid (HL60) or 25 μL ice-cold 50% (w/v) trichloroacetic acid (MCF7) and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 490 nm using Bio-Tek's Elx800 NB 96-well plate reader. The percentage growth was calculated at each of the drug concentration levels based on the difference in OD at the start and end of drug exposure. Values were corrected for background OD from wells only containing medium.
14. Software-predicted lipophilicity of the compounds was calculated with the program CLOGP accessible via Internet (www.daylight.com/daycgi/clogp) working with the Hansch–Leo's ''fragment constant'' method.
15. For a comparison study with other computer programs see: Machatha, S. G.; Yalkowsky, S. H. *Int. J. Pharm.* **2005**, *294*, 185.