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Stereoselective and Improved Syntheses and Anticancer Testing of 3′-*O*-silatranylthymidines

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Abstract—A stereoselective synthesis of 3′-*O*-((*R,R,R*)-trimethylsilatranyl)thymidine (*R,R,R*-**1**) and synthesis of 3′-*O*-silatranylthymidine (**5**) via an improved silatranylation procedure using tetrakis(dimethylamino)silane are reported. Diastereomeric mixture **1** showed more activity than *R,R,R*-**1** or **5** in a primary anticancer screen against breast, CNS, and lung cell lines; demonstrating the import of the configuration and presence, respectively, of the silatrane methyl groups for growth inhibition.
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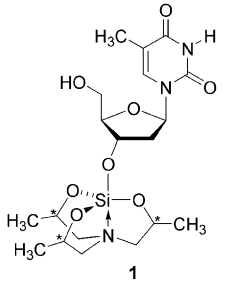
Recently we introduced atranyl-nucleosides as transition state analogues for phosphoryl transfer reactions.[1] Herein we report improved syntheses and anticancer screening results for 3′-*O*-silatranylthymidines. Since silatranes are known to exhibit wide ranging biological activity[2] and nucleoside analogues have a rich history as drugs,[3] it seemed logical to test the biological activity of 3′-*O*-(trimethylsilatranyl)thymidine (**1**).[1] Hence, **1** was sent to the National Cancer Institute (NCI) for anticancer screening through the Developmental Therapeutics Program[4] which assays the ability of a fixed concentration (1.0×10^{-4} M) of a compound to inhibit the growth of human breast, central nervous system (CNS), and lung cancer cell lines over a 48 h period. The results of this primary screen are reported as growth percentages compared to untreated control cells (growth = 100%); the growth percentages for cells treated with thymidine **1** were: breast 54%, CNS 83%, and lung 88%. The NCI considers a compound to be active when the growth percentage in any one of the cell lines is $\leq$32%. These compounds qualify for screening against 60 cancer cell lines over a 5-log dose range. Since **1** is a mixture of four diastereomers, it was expected that each of the individual diastereomers would have different activity, given that it has been understood for nearly a century that stereochemistry influences pharmacological activity.[5] On the other hand, it was also possible that **1** was simply acting as a silicon source, and the silicon was inhibiting cell growth by some unknown mechanism.[6] If the latter were true, then the stereochemistry of **1** would presumably be inconsequential. We therefore wished to obtain and test the activity of a single diastereomer of **1** as well as a derivative without the silatrane methyl groups. Thus, the stereospecific synthesis of **1** with the *R,R,R*-configurations at the three silatrane stereocenters was undertaken. (Separation of the diastereomeric mixture was not deemed feasible because only small quantities of **1** were on hand.) Hydrolytic kinetic resolution[7] of racemic propylene oxide furnished *R*-propylene oxide which was subsequently heated with ammonia in a sealed tube, following the procedure of Nugent and Harlow,[8] to provide *R,R,R*-triisopropanolamine.



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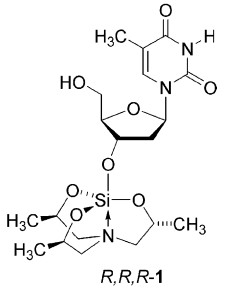

R,R,R-**1**

Silatranylation of 5′-*O*-(4,4′-dimethoxytrityl)-thymidine (**2**) was accomplished by heating with tetraethyl orthosilicate and KOH, followed by addition of the *R,R,R*-triisopropanolamine to give silatrane **3** in 26% yield

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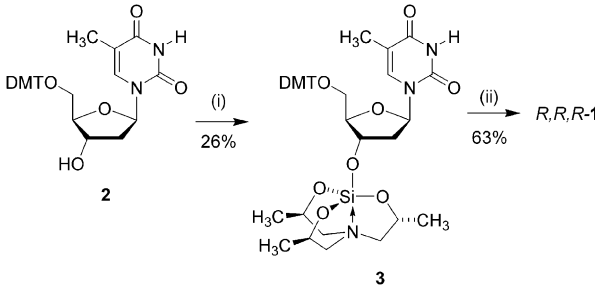
(Scheme 1). *R,R,R*-**1** was then afforded in 63% yield after detritylation with dichloroacetic acid. As fully expected, the spectra[9] of *R,R,R*-**1** are much less complicated than those of the mixture **1**; for example, ^{29}Si NMR of *R,R,R*-**1** shows a single silatrane resonance at −97.56 ppm whereas four silatrane resonances were previously observed in the ^{29}Si NMR spectrum of **1**.[1]

Given the abysmal silatranylation yield, alternative methods were sought in the context of preparing the corresponding silatranylthymidine without the adorning methyl groups; that is, **5**. Alternate silicon sources were thus explored, of which tetrakis(dimethylamino)silane (**6**) proved to be the best. Thus, heating thymidine **2** with excess **6** and triethanolamine in *o*-dichlorobenzene provided silatrane **4** in 51% (Scheme 2). Unfortunately, **6** is only intermittently available through commercial suppliers[10] and its synthesis is intractable.[11] Therefore, yet another silatranylation procedure was developed. Commercially available boratrane was converted to 1-hydrosilatrane[12] which was then dehydrogenatively coupled[13] with thymidine **2** using Wilkinson's catalyst [$ClRh(PPh_3)_3$] to afford silatrane **4** in slightly lower yield (41%). Cobalt carbonyl [$Co_2(CO)_8$] also promoted the dehydrogenative coupling, however in our hands the yields with this catalyst were less consistent. Removal of the dimethoxytrityl protecting group from **4** under the conventional conditions (2.5% $CHCl_2CO_2H/CH_2Cl_2$) offered only low yields of silatranylthymidine **5**. Instead, the best deprotection conditions resulted from refluxing **4** with K 10 montmorillonite[14] in methanol, which furnished a 77% yield of silatranylthymidine **5**.[15]

The anticancer activity of *R,R,R*-**1** and **5** were determined at the NCI (Table 1). The import of the configuration of the silatrane methyl groups is immediately apparent. *R,R,R*-**1** showed no activity in the breast and lung cell lines and actually promoted the growth of the CNS cancer cells. These results rule out the possibility that **1** was simply acting as a silicon source (*vide supra*) and suggest that the activity observed with **1** resulted from one (or more) of the three remaining stereoisomers. Alternatively, it could also be postulated that all isomers are required for a synergistic effect. Current work towards the synthesis and screening of each of the remaining diastereomers will resolve this issue. If all the activity does reside with one isomer, it is possible that when tested alone it will drop the cell growth below the 32% threshold and qualify for the 60-cell line screening (*vide supra*). In the case of silatranylthymidine **5**, no activity is observed in the CNS and lung cells and only 19% growth inhibition is seen in the breast cell line. Two possible explanations are offered for the increased inhibition observed with **1** compared to **5**. Voronkov and co-workers[16] have shown that silatranes adorned with methyl groups are more stable towards hydrolysis than those without; consequently, much of **5** may simply decompose prior to reaching its putative target. Alternatively, the methyl groups of *R,R,S*-**1**, *S,S,R*-**1**, and/or *S,S,S*-**1** may enhance binding with the putative target.

Preliminary antiviral testing of diastereomeric mixture **1** and **5** was conducted as arranged through the National Institute of Allergy and Infectious Diseases (NIAID). Thus, **1** showed no activity ($EC_{50} > 100$ μg/mL) against herpes simplex virus type 1 (HSV1) or type 2 (HSV2) as measured by a cytopathic effect inhibition assay with human foreskin fibroblast (HFF) cells infected with HSV.[17] Testing of **5** against hepatitis B virus in HBV-producing human liver cell line 2.2.15 also showed no activity ($EC_{50} > 10$ μM) and relatively low toxicity ($CC_{50} = 1294$ μM, standard devation = 32).[18,19]

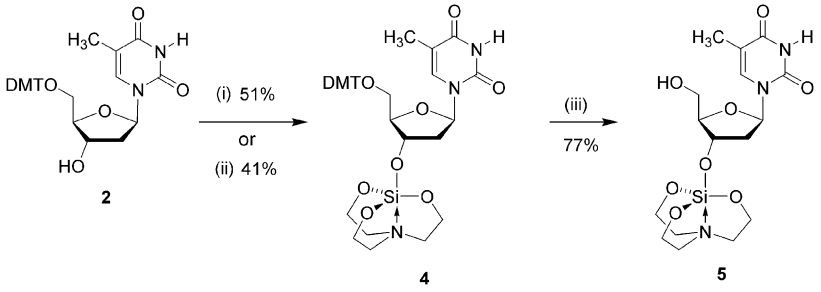


Scheme 1. (i) 4.0 equiv $Si(OCH_2CH_3)_4$, 4.0 equiv *R,R,R*-triisopropanolamine, 1.0 equiv KOH, DMF, 125 °C, 96 h; (ii) 2.5% $CHCl_2CO_2H/CH_2Cl_2$, rt, 3.5 min.

Table 1. Growth percentages[a,b] of cancer cell lines after a 48 h incubation with compounds **1**, *R,R,R*-**1**, and **5**

Compd	Breast cell line (MCF7)	CNS cell line (SF-268)	Lung cell line (NCI-H460)
1	54	83	88
R,R,R-**1**	103	129	101
5	81	97	98

[a]Compared to untreated control cells where growth is defined as 100%.
[b]Compound concentrations = 1.0×10^{-4} M.



Scheme 2. (i) 5.0 equiv $Si(NMe_2)_4$, 10.0 equiv $N(CH_2CH_2OH)_3$, *o*-dichlorobenzene, 155 °C, 25 h; (ii) 2.5 equiv $HSi(OCH_2CH_2)_3N$, 0.3 equiv $ClRh(PPh_3)_3$, xylenes, 115 °C, 19 h; (iii) K 10 Clay, CH_3OH (reflux), 80 min.

In conclusion, a stereoselective synthesis of 3′-*O*-((*R,R,R*)-trimethylsilatranyl)thymidine (*R,R,R*-**1**) and improved silatranylation conditions in the synthesis of 3′-*O*-silatranylthymidine (**5**) are reported. Anticancer testing reveals that the methyl groups are required for the best activity and that the configuration of the methyl groups is important. Synthesis and anticancer testing of *R,R,S*-**1**, *S,S,R*-**1**, and *S,S,S*-**1** will determine if the growth inhibition observed for the mixture **1** is derived from a single diastereomer.

Acknowledgements

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