Inhibition of Vertebrate Squalene Epoxidase by Extended and Truncated Analogues of Trisnorsqualene Alcohol

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The epoxidation of squalene to (3S)-2,3-epoxysqualene and subsequent cyclization to lanosterol are keys steps in vertebrate cholesterol biosynthesis. Trisnorsqualene alcohol (TNSA) has previously been reported as a potent inhibitor of vertebrate squalene epoxidase, with IC_{50} = 4 μM for pig liver (*J. Am. Chem. Soc.* **1989**, *111*, 1508-1510). Analogues with extended and truncated carbon skeletons have been prepared and tested for pig liver squalene epoxidase (SE) inhibition. Most of the structural analogues were poor inhibitors of vertebrate SE, with the exception of bisnorsqualene alcohol which had the same activity as TNSA. These results support the theory that an intact trisnorsqualene moiety is required for activity.

Squalene epoxidase (EC 1.14.99.7) catalyzes the stereospecific conversion of squalene to (3S)-2,3-epoxysqualene and is a key enzyme in cholesterol biosynthesis.[1] While drugs inhibiting HMG-CoA reductase are effective in lowering blood serum cholesterol levels,[2] we[3] and others[4] have examined the inhibition of squalene epoxidase and oxidosqualene cyclase as potential therapeutic targets. Efforts to develop inhibitors of squalene epoxidase (SE) have led to the discovery of a class of allylamines at Sandoz (e.g., terbinafine and naftifine) which inhibit fungal SE with IC_{50} values in the 30 nM range.[5] These compounds are effective antifungal agents, since they show little inhibition of vertebrate SE even at 30 μM.[6] 2-Aza-2,3-dihydrosqualene, a potent inhibitor of vertebrate oxidosqualene cyclase, has also been found to inhibit rat liver SE.[7] Recently, we described the inhibition of pig liver SE by trisnorsqualene alcohol (TNSA).[8] We now report the preparation of homologues and isoprenologues of TNSA, and we describe their potency as in vitro inhibitors of pig liver SE.

Results and Discussion

The TNSA analogues to be prepared and tested for pig liver SE inhibition are shown in Schemes I and II and include isoprenologues **2**, **3**, and **4**, and homologues **5** and **6** of TNSA. The TNSA analogues containing varying isoprene units were synthesized as shown in Scheme I. Truncated analogues **2** and **3** were prepared by $NaBH_4$ reduction of the corresponding aldehydes, obtained from periodic acid cleavage of a mixture of 10,11- and 6,7-epoxysqualene.[9] The extended isoprenologue **4** was obtained by the addition of isopropenylmagnesium bromide to trisnorsqualene aldehyde,[10] followed by Claisen orthoester rearrangement[11] and reduction of the homoallylic ester.

Analogues **5** and **6** were prepared by $NaBH_4$ reduction of tetranor- and bisnorsqualene aldehydes, respectively, obtained by using the procedure of van Tamelen et al. (Scheme II).[12] Thus, trisnorsqualene aldehyde was converted to the piperidine enamine and then reacted with trimethylene bis(thiotosylate). Enamine hydrolysis, base-induced aldehyde elimination of the resulting α-(dithioketal) aldehyde, and finally deprotection provided tetranorsqualene aldehyde. Reduction with sodium borohydride provided tetranorsqualene alcohol **5**. The one-carbon-extended enol ether obtained by Wittig coupling of trisnorsqualene aldehyde with lithium (methoxymethylidene)triphenylphosphorane, was hydrolyzed to give bisnorsqualene aldehyde. The hydrolysis conditions were optimized by using glacial acetic acid (25 equiv) in a 2:1 ratio of glyme in water solution, at 60 °C for 48 h;[13] other acidic conditions led to a complex mixture of partially cyclized products. Reduction of this aldehyde provided the bisnorsqualene alcohol **6**.

Table I. IC_{50} of TNSA (1) and Analogues 2-6 (Compounds with IC_{50} > 400 μM Showed Slight Inhibition at High [I])

TNSA analogue	IC_{50}, μM
1	4
2	100
3	>400
4	>400
5	40
6	4

The results of the inhibition of pig liver squalene epoxidase by TNSA and its analogues are shown in Table I. None of the analogues prepared was a better inhibitor than TNSA. Addition and removal of isoprene units present in the trisnorsqualenoid moiety resulted in a dramatic decrease in inhibitory potency. Thus, alcohol **3**, which lacks two isoprene units, and alcohol **4**, which has

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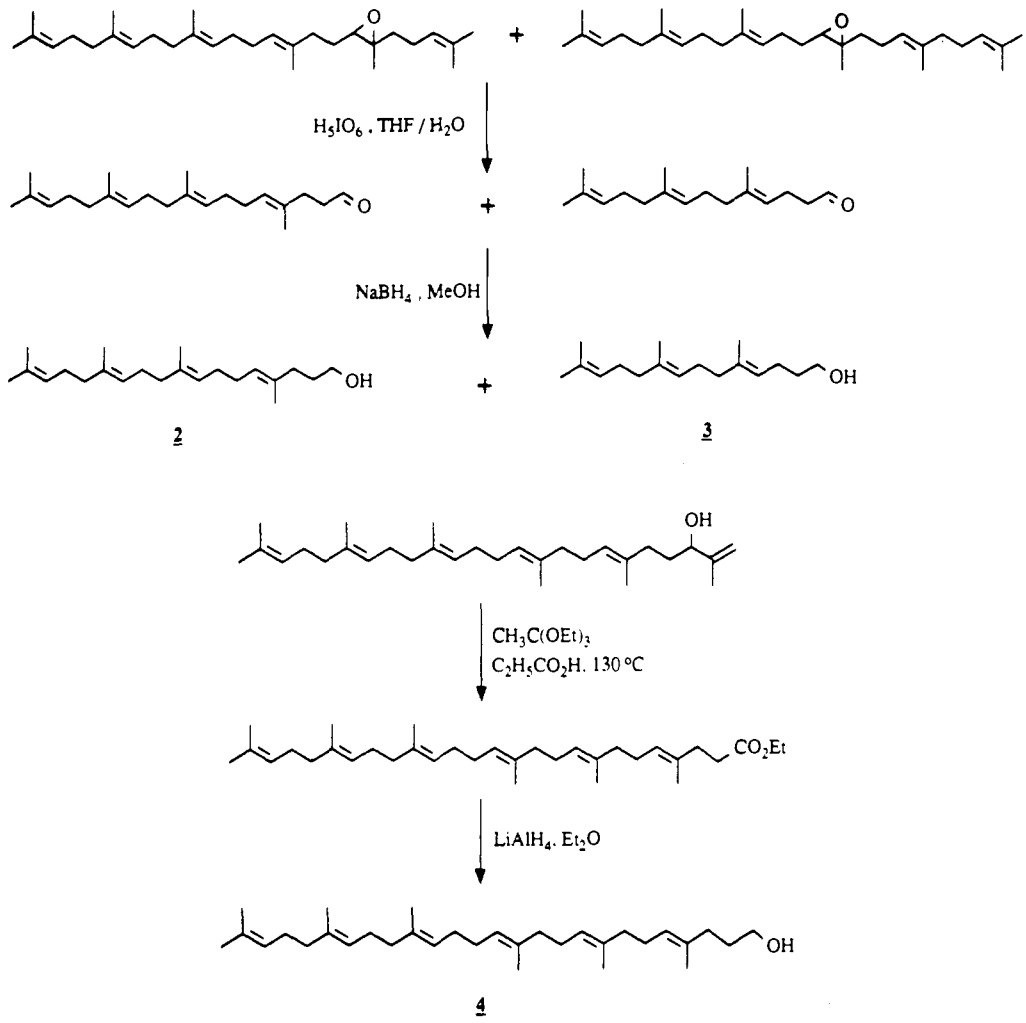
Scheme I. Synthesis of Trisnorsqualene Alcohol Isoprenologues 2 through 4



one additional isoprene unit, showed essentially no activity at concentrations of 400 μM. Even isoprenologue **2**, which lacks only the terminal isopropylidene region of TNSA, was a 25-fold worse inhibitor than TNSA. These results support the theory that the terminal isoprene unit is essential in binding of substrate to enzyme. Previous studies by van Tamelen and Heys on the enzymatic epoxidation of squalene analogues, have shown that the 24,25-dihydro analogue of squalene and smaller isoprenoids were poor substrates for vertebrate squalene epoxidase.[14]

While tetranorsqualene alcohol (**5**) showed a 10-fold decrease in SE inhibition, the activity of bisnorsqualene alcohol **6** was comparable to that of TNSA. It is possible that the hydroxyl groups of TNSA and primary alcohol **6** adopt the same spatial orientation when binding to the enzyme, perhaps mimicking the transition state of epoxide formation (Scheme III). This steric flexibility in the alcohol region is unexpected, as secondary alcohols TNS methyl carbinol and TNS isopropyl carbinol were 10–100-fold less potent inhibitors of pig liver SE.[8,15]

Experimental Section

Mixture of (4*E*,8*E*,12*E*)-4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraenal and (4*E*,8*E*)-5,9,13-Trimethyl-4,8,12-tetradecatrienal.[9] To a 1:1 mixture of internal squalene 6,7- and 10,11-monoepoxides (2.5 g, 6 mmol) in THF (36 mL) were added water (9 mL) and periodic acid (1.5 g, 1.1 equiv). The reaction mixture was stirred for 6 h at room temperature, then diluted with diethyl ether, and washed (saturated $NaHCO_3$, brine). The combined organic extracts were dried ($MgSO_4$) and concentrated. Flash chromatography using 2% ethyl acetate in hexane (EA/H) afforded a 1:1 mixture of the two polyene aldehydes which were used without separation (1.15 g, 80%).

Mixture of (4*E*,8*E*,12*E*)-4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraen-1-ol and (4*E*,8*E*)-5,9,13-Trimethyl-4,8,12-tetradecatrien-1-ol (2 and 3). To a solution of the above tetraenal and trienal mixture (0.27 g, 1 mmol) in CH_3OH (5 mL) was added $NaBH_4$ (0.038 g, 1 equiv). The reaction mixture was stirred for 0.5 h and concentrated in vacuo, and the residue was dissolved in Et_2O. The ethereal solution was washed (brine), and the combined organic extracts were dried ($MgSO_4$) and concentrated. The homologous alcohols were separated by reverse phase chromatography (C_{18}/Porasil B, 30 μm) by slow elution with 70:30 CH_3–H_2O. The collected fractions containing the separated alcohols were dissolved in ethyl ether and washed (brine), and the organic layer was dried ($MgSO_4$) and concentrated. The alcohols were rechromatographed (5% EA/H) to give pure tetraenol **2** and trienol **3** (121 mg and 103 mg, respectively, total yield: 83%).

Trienol: IR (neat) 3331, 2926, 1667 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60, 1.61 (s, 9 H, C-15, C-16, C-17 CH_3), 1.67 (s, 3 H, C-14 CH_3), 1.97–2.08 (br m, 12 H, C=CCH_2, C-2 CH_2), 3.63 (t, J = 6.5 Hz, 2 H, CH_2OH), 5.10–5.15 (br m, 3 H, C=C*H*); ^{13}C NMR ($CDCl_3$) δ 15.90, 17.58 (C-15, C-16, C-17), 24.19 (C-2*), 25.60 (C-14*), 26.50, 26.68 (C-3, C-7, C-11), 32.69, 39.65 (C-6, C-10), 62.55 (C-1), 123.70, 124.06, 124.32 (C-4, C-8, C-12), 131.14 (C-13), 134.91, 135.59 (C-5, C-9).

Tetraenol: IR (neat) 3327, 2922–2854, 1666 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.59, 1.61 (s, 12 H, C-19, C-20, C-21, C-22 CH_3), 1.67

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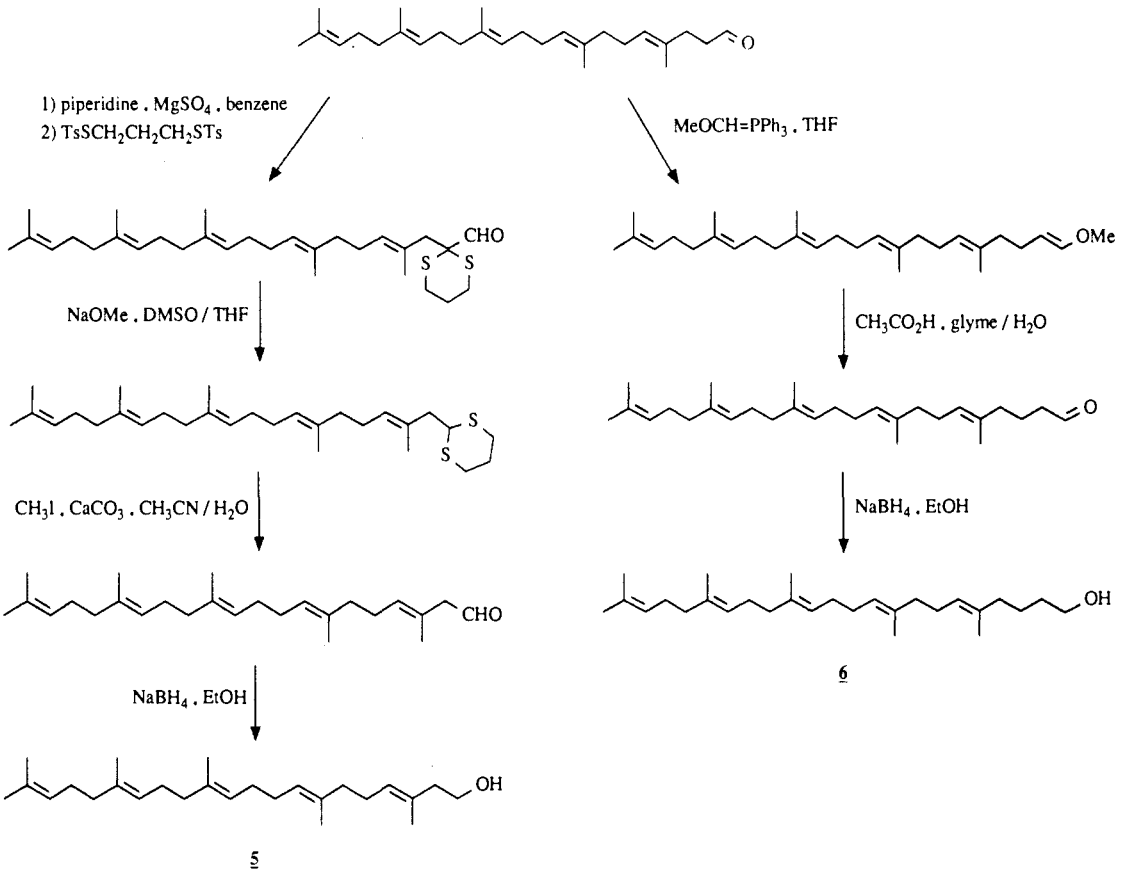
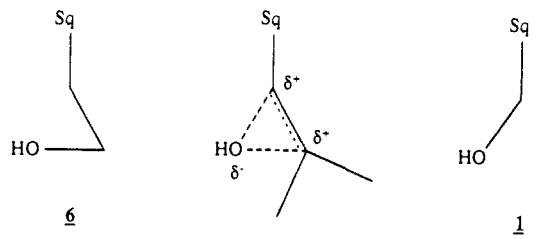
Scheme II. Synthesis of Trisnorsqualene Alcohol Homologues 5 and 6



Scheme III. Possible Orientations for Binding of TNSA (1) and Homologue 6



(s, 3 H, C-18 CH_3), 1.99–2.08 (br m, 16 H, C=CCH_2, C-2 CH_2), 3.62 (t, J = 6.5 Hz, 2 H, CH_2OH), 5.09–5.18 (br m, 4 H, C=C*H*); ^{13}C NMR ($CDCl_3$) δ 62.80 (C-1).

(6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol. To a solution of isopropenylmagnesium bromide, formed from magnesium metal (62 mg, 2.6 mmol) and 2-bromopropene (0.27 g, 2.2 mmol) in THF (7 mL), was added trisnorsqualene aldehyde[11] (0.65 g, 1.7 mmol) in THF (5 mL) at −35 °C. The reaction mixture was slowly warmed to room temperature for 1 h. The reaction mixture was extracted with ether, and the organic extracts were washed (10% $NaHCO_3$, brine), dried ($MgSO_4$), and concentrated. Purification by flash chromatography (5% EA/H) gave pure allylic alcohol (0.55 g, 76%): IR (neat) 3350, 2921–2853, 1667, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60, 1.61 (s, 15 H, C-25, C-26, C-27, C-28, C-29 CH_3), 1.67 (s, 3 H, C-24 CH_3), 1.72 (s, 3 H, CH_2=CCH_3), 2.00–2.06 (br m, 20 H, CH_2), 4.04 (m, 1 H, C*H*OH), 4.83, 4.93 (m, 2 H, C=CH_2), 5.07–5.25 (br m, 5 H, C=C*H*); ^{13}C NMR ($CDCl_3$) δ 75.64 (C-3), 110.94 (C-1), 147.48 (C-2).

Ethyl (4*E*,8*E*,12*E*,16*E*,20*E*)-4,8,12,17,21,25-Hexamethyl-4,8,12,16,20,24-hexacosahexaenoate. To a solution of the above alcohol (514 mg, 1.2 mmol) in ethyl orthoacetate (3.5 mL) was added propionic acid (15 μL). The solution was warmed to 138 °C for 1 h. The reaction mixture was then diluted with hexane and washed (10% $NaHCO_3$, water), and the resulting organic extract was dried ($MgSO_4$) and concentrated. Flash chromatography (1.5% EA/H) of the resulting oil gave pure ester (0.48 g, 80%): IR (neat) 2966–2854, 1738, 1667 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (t, J = 7.1 Hz, 3 H, C(O)CH_2CH_3), 1.60 (s, 18 H, C-27, C-28, C-29, C-30, C-31, C-32 CH_3), 1.67 (s, 3 H, C-26 CH_3), 2.00–2.06 (br m, 22 H, C=CCH_2), 2.32–2.38 (br m, 4 H, C-2, C-3 CH_2), 4.11 (q, J = 7.1 Hz, 2 H, C(O)CH_2CH_3), 5.09–5.22 (br m, 6 H, C=C*H*); ^{13}C NMR ($CDCl_3$) δ 14.24 (C(O)CH_2CH_3), 34.70 (C-2*), 39.56 (C-3*), 60.16 (C(O)CH_2CH_3), 173.46 (*C*=O).

(4*E*,8*E*,12*E*,16*E*,20*E*)-4,8,12,17,21,25-Hexamethyl-4,8,12,16,20,24-hexacosahexaen-1-ol (4). To a solution of the above ethyl ester (423 mg, 0.85 mmol) and Et_2O (5 mL) was added $LiAlH_4$ (32 mg, 1 equiv, in 5 mL of Et_2O). The reaction mixture was stirred for 0.5 h and then quenched by the addition of saturated NH_4Cl. The solution was extracted with ether, and the organic layer was washed (brine), dried ($MgSO_4$), and concentrated. The resulting oil was purified by flash chromatography (10% EA/H) to give pure 4 (312 mg; 85%): IR (neat) 3333, 2921–2853, 1667 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60, 1.65 (s, 18 H, C-27, C-28, C-29, C-30, C-31, C-32 CH_3), 1.67 (s, 3 H, C-26 CH_3), 1.98–2.09 (br m, 24 H, C=CCH_2, C-2 CH_2), 3.63 (t, J = 6.5 Hz, 2 H, C-1 CH_2OH), 5.09–5.17 (br m, 6 H, C=C*H*); ^{13}C NMR ($CDCl_3$) δ 62.67 (C-1).

(3*E*,7*E*,11*E*,15*E*)-3,7,12,16,20-Pentamethyl-3,7,11,15,19-heneicosapentaen-1-ol (5). To a solution of crude tetranorsqualene aldehyde[12,13] (20 mg, 0.06 mmol) in ethanol (7 mL) was added $NaBH_4$ (2 mg, 1 equiv). The solution was stirred at room temperature for 1 h and then quenched by the addition of 2 N CH_3CO_2H. The reaction mixture was subjected to the usual work-up procedures and purified by flash chromatography (10–20% EA/H gradient) to afford pure tetranorsqualene alcohol 5 (8 mg, 40%, R_f = 0.31, 20% EA/H): IR (neat) 3354.4, 1665.3 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60 (br s, 12 H, C-22, C-23, C-24, C-25 CH_3), 1.63 (s, 3 H, C-26 CH_3), 1.68 (s, 3 H, C-21 CH_3), 2.00 (br m, 16 H, C-5, C-6, C-9, C-10, C-13, C-14, C-17, C-18 CH_2), 2.24 (t, J = 6 Hz, 2 H, C-2 CH_2), 3.63 (t, J = 6 Hz, 2 H, CH_2OH), 5.14 (br m, 5 H, C=C*H*). Anal. Calcd: C, 83.80; H, 11.90. Found: C, 83.76; H, 11.88.

(5*E*,9*E*,13*E*,17*E*)-5,9,14,18,22-Pentamethyl-5,9,13,17,21-tricosapentaen-1-ol (6). Alcohol 6 was obtained from bisnor-

squalene aldehyde[3] by using the same procedure as above (14 mg, 29%, R_f = 0.24, 20% EA/H): IR (neat) 3343.1, 1666.4 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (quintet, J = 6.6 Hz, 2 H, C-3 CH_2), 1.60 (br s, 15 H, C-24, C-25, C-26, C-27, C-28 CH_3), 1.68 (s, 3 H, C-23 CH_3), 2.00 (br m, 20 H, C-2 CH_2 and C=CCH_2), 3.64 (t, J = 6.3 Hz, 2 H, CH_2OH), 5.12 (br m, 5 H, C=CH); ^{13}C NMR ($CDCl_3$) δ 15.82, 16.02, 17.66 (C-24, C-25, C-26, C-27, C-28), 24.00 (C-23), 25.68 (C-3), 26.62, 26.65, 26.77 (C-7, C-16, C-20), 28.26 (C-11, C-12), 32.32 (C-2), 39.32, 39.73 (C-4, C-8, C-15, C-19), 62.98 (C-1), 124.27, 124.39, 124.55 (C-6, C-10, C-13, C-17), 131.23 (C-22), 134.69, 134.88, 135.03, 135.10 (C-5, C-9, C-14, C-18). Anal. Calcd: C, 83.83; H, 12.08. Found: C, 83.94; H, 12.27.

IC_{50} Determinations for Inhibition of SE and OSC. Test tubes containing either squalene epoxidase or oxidosqualene cyclase enzyme solutions[3] (240 μL each) were warmed to 37 °C. After 10 min, squalene analogues were added (1 μL in 2-propanol) to give final inhibitor concentrations of 0, 4, 20, 40, 200, and 400 μM. After an additional 10 min, [^{14}C]squalene (2 μL in 2-propanol, ca. 20000 dpm, 33 μM)[3] was added to each enzyme solution. Incubation was continued for another 50 min and then stopped by the addition of 10% KOH/methanol (240 μL). After 1 h at 37 °C, each mixture was extracted with CH_2Cl_2 (1 mL each); the resulting organic extracts were dried ($MgSO_4$) and redissolved in a small amount of CH_2Cl_2 (100 μL), and the triterpene components were separated by thin-layer chromatography. Radiochemical analysis, using either linear analysis or scintillation counting, showed conversion of squalene to either 2,3-epoxysqualene (for the epoxidase assays), or a mixture of lanosterol and 2,3-epoxysqualene (for the cyclase assays). Inhibitor-free assays showed approximately 30% conversion to product, and radiochemical recoveries of 80%-90% were routinely achieved.

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All Atom Molecular Mechanics Simulations on Covalent Complexes of Anthramycin and Neothramycin with Deoxydecanucleotides

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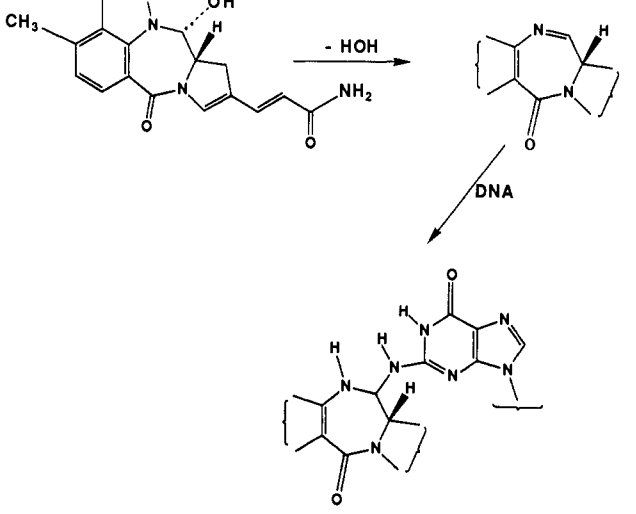
We present molecular mechanics simulations on covalent complexes between d[$(GC)_5$]$_2$, d(G_{10})·d(C_{10}), d-(GCGCGAGCGC)·d(GCGCTCGCGC), d(GCGCGTGCGC)·d(GCGCACGCGC), d(G_5AG_4)·d(C_4TC_5), and d-(G_5TG_4)·d(C_4AC_5) on one hand and potent antitumor antibiotics anthramycin and neothramycin A on the other, using the all atom force field in the framework of the program AMBER(UCSF). The energy-refined models of both the sets of complexes show minimal distortions for the nucleotides, consistent with the results of 2D NMR studies on these complexes. The drugs have 3'-orientation in the minor groove, consistent with the previously reported investigations employing the united atom force field and with the experimental observations. Both anthramycin and neothramycin are calculated to bind preferentially to the puGpu sequences over pyGpy. This is in qualitative agreement with experimental studies for anthramycin, while for neothramycin A, this result is in apparent disagreement with experimental observations which have reported preferential binding of neothramycin A to poly(dG-dC)·poly(dG-dC) over poly(dG)·poly(dC). While the present study brings out the usefulness of the simple molecular mechanics approach (using an all atom force field) in rationalizing substantial experimental observations, it also emphasizes the need for further investigations on solvent and dynamics effects in understanding the sequence specificity of drug-DNA binding.

Pyrrolo[1,4]benzodiazepines (PBDs) (Figure 1) are potent antitumor antibiotics derived from various streptomyces species[1,2] and act by binding in the minor groove of DNA and alkylating it on the 2-amino group of a guanine residue.[3-8] This groove binding is facilitated by a twist in the PBD structure, fixed by the chiral center at C11a, that gives it a precise fit to right-handed B-DNA.[9,10] As shown in Scheme I, the DNA alkylation occurs at C11 of the PBD. This carbon occurs in a variety of substitution patterns: carbinolamine, carbinolamine ether, or imine, but all of them are potential alkylating groups. The precise mechanism or mechanisms of alkylation are not defined at this time; however, it is possible that initial noncovalent binding, stabilize by a network of hydrogen bonds and van der Waals interactions, positions the molecule for subsequent covalent bond formation. Computer modeling indicates that this is a reasonable scenario.[11] Detailed structures of the covalent complexes have been provided by NMR studies,[7,12] some of which were made in conjunction with computer modeling.[13,14]

The sequence specificity for binding of PBDs to double helical DNA is thought to be important in their antitumor activity.[15,16] It also is fundamental to their potential use as DNA probes. An early study on the binding of an-

Scheme I. Alkylation of DNA (2-Amino Group of G) at C11 of a Typical PBD



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