# Stereostructures of Reaction Products of Thymidine Epoxides with Amines and L-Amino Acid Ethyl Esters

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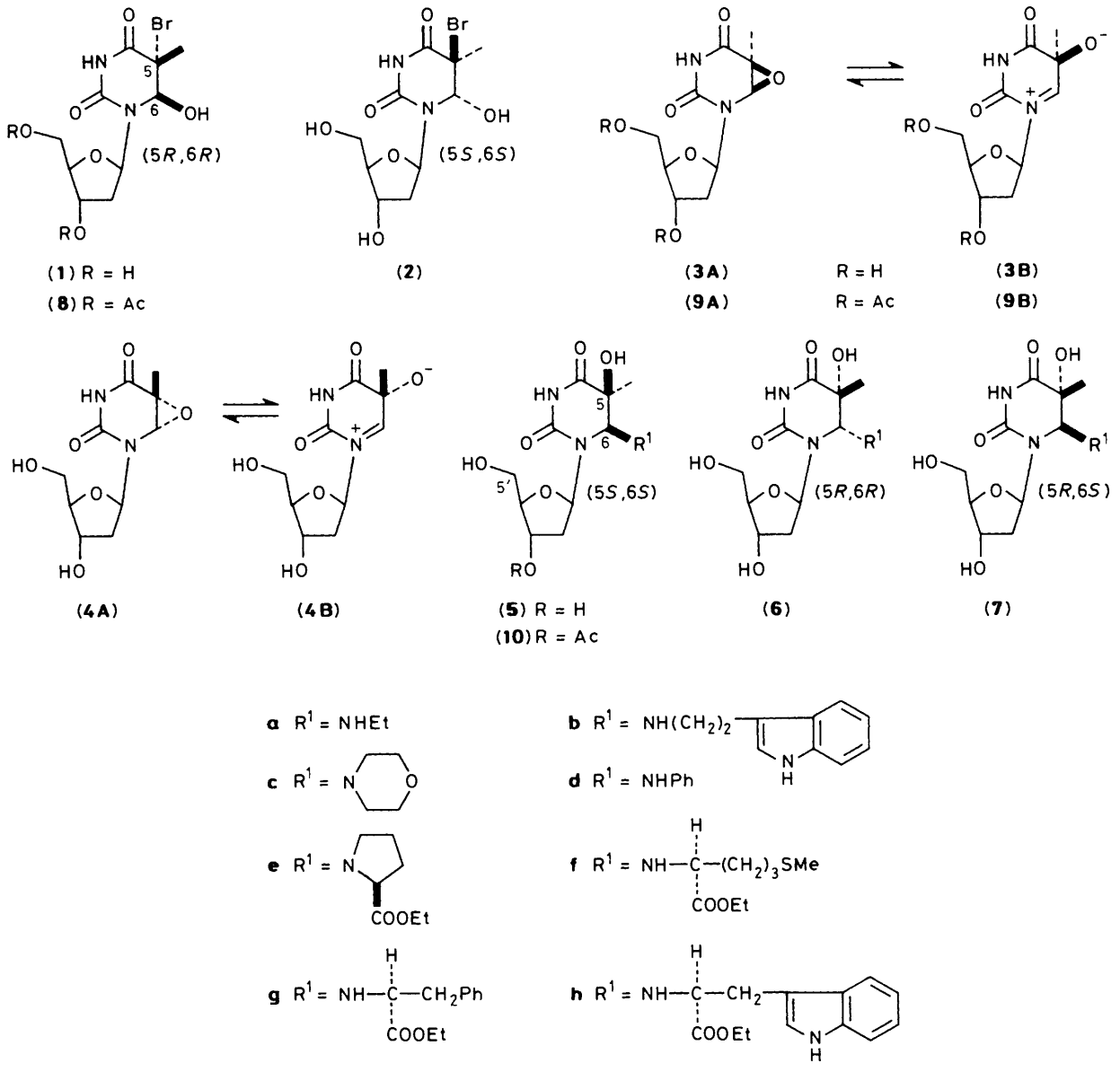
Reaction of thymidine epoxides, (**3**) and (**4**), with amines and L-amino acid ethyl esters was investigated and the stereostructures of products were determined.

It is well known that active oxygen species including the hydroxyl radical, superoxide anion, hydrogen peroxide, and singlet oxygen are generated in living cells. The oxidation reactions of nucleic acids and related compounds by reactive oxygen species or other oxidising biocomponents such as lipid hydroperoxides have recently received much attention in connection with their possible involvement in mutagenesis, carcinogenesis, and aging.[1—6] It has been reported that the *cis* isomers of thymine and thymidine glycols are released in human and rat urines as the result of the repair of oxidatively damaged DNA.[7] It should also be considered that the intermediates of these oxidation reactions may also react with nucleophiles such as amino acids or purine and pyrimidine nucleic acid components. Therefore, we decided to investigate the reaction of thymidine epoxides, (**3A**) and (**4A**), and/or their equivalents, (**3B**) and (**4B**), which may be the precursors of the *cis* thymidine glycol mentioned above, with several amines and L-amino acid derivatives as a model reaction for nucleic acid–protein cross-links. Reaction of (**3**) prepared *in situ* from (+)-*trans*-(5*R*,6*R*)-5-bromo-6-hydroxy-5,6-dihydrothymidine (**1**)† and triethylamine with nucleophiles in tetrahydrofuran (THF) at room temperature gave the cross-coupling product (**5**)‡ in high yield (see Table 1). On the other hand, reaction of (**4**) prepared from (−)-*trans*-(5*S*,6*S*)-5-bromo-6-hydroxy-5,6-dihydrothymidine (**2**) under similar experimental conditions gave two cross-coupling products, (**6**) and (**7**)‡ (see Table 2). The isomerisation procedure was applied to the products in order to investigate their stereostructures.[9,10] Thus, treatment of (**5a—h**) with $BF_3$·$Et_2O$ in

† Reaction of thymidine with *N*-bromosuccinimide (NBS) in $H_2O$ under ice-cooling afforded (**1**) {$[\alpha]_D$ +49.7° (*c* 2.0 in $H_2O$)} and (**2**) {$[\alpha]_D$ −43.0° (*c* 1.0 in $H_2O$)}, in 66 and 31% yields, respectively. These stereostructures, including absolute configuration, were determined by direct comparison with authentic samples prepared by the bromine method.[8]

‡ All new compounds gave satisfactory n.m.r. and i.r. spectra and elemental analysis and/or mass spectra.




THF at room temperature caused no isomerisation but resulted in the recovery of starting materials in moderate to good yield, suggesting that (**5a**—**h**) were the *cis* products. This assumption was confirmed by an *X*-ray analysis of (**5a**) as shown in Figure 1.§ Therefore, the stereostructures of the *cis* products from (**1**) can be represented by formulae (**5a**—**h**) with 5*S* and 6*S*, supporting the mechanism of substitution of the bromine atom in (**1**) which involves an $S_N2$ type reaction with the participation of the vicinal 6-hydroxy group.[11] Treatment of (**6a**—**h**) with $BF_3{\cdot}Et_2O$ resulted in the recovery of the starting materials in moderate to good yield, while (**7c**—**g**) were very labile to treatment with $BF_3{\cdot}Et_2O$, affording the isomerisation products (**6c**—**f**) in poor yield along with unidentified products. These facts suggest that (**6a**—**h**) are the *cis* products and (**7c**—**g**) are the corresponding *trans* isomers. Moreover we have recently reported that the diastereoisomers with a (5*R*,6*R*) stereoconfiguration are more *laevorotatory* than the (5*R*,6*S*) isomers obtained from the reaction of (±)-1,3-dimethylthymine epoxide with L-amino acid ethyl esters.[10] Therefore, it would be reasonable to assume that (**6**), which is more *laevorotatory*, has a (5*R*,6*R*) configuration whereas (**7**) has a (5*R*,6*S*) configuration. Consequently, among the products derived from (**2**), the stereostructures of the (5*R*,6*R*) *cis* products can be represented by formulae (**6a**—**h**) and those of the (5*R*,6*S*) *trans* products by formulae (**7c**—**g**), respectively.

The reason why (**3**) prepared from (**1**) gives a single product

§ *Crystal data for* (**5a**): $C_{12}H_{21}N_3O_6$, $M$ = 303.14, monoclinic, space group $P2_1$, $a$ = 6.209(0), $b$ = 14.977(1), $c$ = 8.180(1) Å, β = 109.42(1)°, $U$ = 717.4 Å$^3$, $Z$ = 2, and $D_c$ = 1.404 g cm$^{-3}$. The reflection data were collected on a Rigaku AFC-5 diffractometer for $0 < \theta < 60°$ using monochromated Cu-$K_\alpha$ radiation and $\omega$–2$\theta$ scan technique. The structure was solved by direct methods and refined by full-matrix least-squares. The final $R$ value was 0.042 for 1074 independent reflections [$F > 3\sigma(F)$].

Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1, 1986.

**Table 1.** The results of reaction of (**3**) with nucleophiles.[a]

| Nucleophile | Product | Yield % | Reaction time | $[\alpha]_D$ (*c* 1.0 in EtOH) |
|---|---|---|---|---|
| Ethylamine | (**5a**) | 98.4 | 20 min | +20.1° |
| Tryptamine | (**5b**) | 95.0 | 20 h | +19.5° |
| Morpholine | (**5c**) | quant. | 20 h | +29.1° |
| Aniline | (**5d**) | 61.6 | 18 h | +37.3° |
| Pro-OEt | (**5e**) | 97.3 | 18 h | −25.1° |
| Met-OEt | (**5f**) | 96.6 | 24 h | +4.3° |
| Phe-OEt | (**5g**) | 81.0 | 24 h | +13.1° |
| Trp-OEt | (**5h**) | 98.7 | 24 h | +16.7° |

[a] Carried out by using 1.5 equiv. of triethylamine and 20 equiv. of nucleophile except ethylamine, tryptamine, and morpholine (each 2 equiv.).

**Table 2.** The results of reaction of (**4**) with nucleophiles.[a]

| Nucleophile | Product | Yield % (Total yield) | Reaction time | $[\alpha]_D$ (*c* 1.0 in EtOH) |
|---|---|---|---|---|
| Ethylamine | (**6a**) | 53.3 | 20 min | −20.7° |
| | (**7a**) | [b] | | — |
| Tryptamine | (**6b**) | 53.4 | 16 h | −23.1° |
| | (**7b**) | [b] | | — |
| Morpholine | (**6c**) | 34.3 } 81.0 | 18 h | −1.1° |
| | (**7c**) | 46.7 } | | +72.9° |
| Aniline | (**6d**) | 22.0 } 62.8 | 42 h | +26.3° |
| | (**7d**) | 40.8 } | | +91.3° |
| Pro-OEt | (**6e**) | 31.7 } 63.3 | 40 h | −69.2° |
| | (**7e**) | 31.6 } | | +12.3° |
| Met-OEt | (**6f**) | 32.1 } 44.2 | 45 h | −65.0° |
| | (**7f**) | 12.1 } | | +10.5° |
| Phe-OEt | (**6g**) | 36.8 } 60.9 | 45 h | −43.9° |
| | (**7g**) | 24.1 } | | +22.1° |
| Trp-OEt | (**6h**) | 31.5 | 45 h | −34.6° |
| | (**7h**) | [b] | | — |

[a] Carried out by using 1.5 equiv. of triethylamine and 20 equiv. of nucleophile except for ethylamine (2 equiv.). [b] Not isolable in a pure state because of its instability.

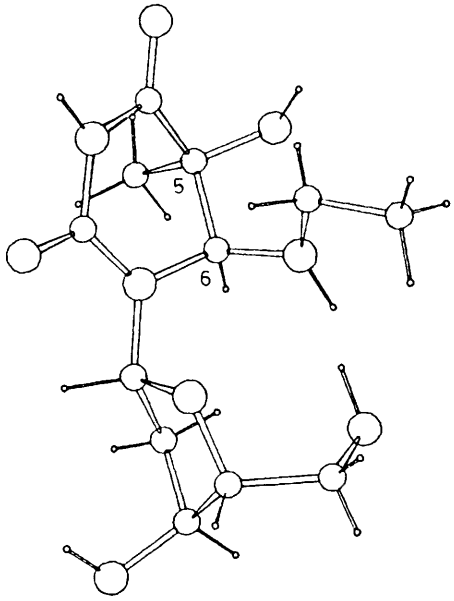



**Figure 1.** Molecular structure of (**5a**).

(*cis* product) remains unclear. However, the 5′-hydroxy group does not participate in forming a single product, since reaction of 3′,5′-diacetyl bromohydrin (**8**)[8] with morpholine *via* (**9**) yielded a single product (**10c**),‡ which was identical with an acetylation product of (**5c**). The preferential formation of the *cis* products may be attributable to a main contribution of the ionic intermediates, (**3B**) and (**4B**), in reaction of thymidine epoxides, (**3**) and (**4**), with nucleophiles.[9,12] The present results may be useful for a better understanding of the chemistry of the cross-linking products of nucleic acid with protein *in vivo*.

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