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SYNTHESIS OF N^6-ADENOSINE ADDUCTS EXPECTED FROM CYCLOPENTA-RING ACTIVATION OF ACENAPHTHYLENE AND ACEANTHRYLENE

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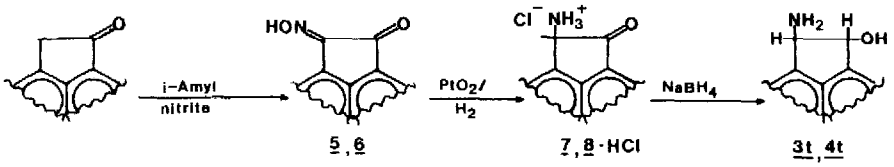
ABSTRACT: cis and trans Acenaphthen-1-amine-2-ol and trans aceanthren-1-amine-2-ol react with 2-chloropurine-9-β-D-ribofuranose to yield easily separable diastereomeric mixtures of N^6-modified adenosine, corresponding to RNA adducts expected from the corresponding 1,2-oxides. These are the first examples of nucleoside adducts of cyclopentaPAH.

Although a number of polycyclic aromatic systems containing peripherally fused cyclopenta rings (cyclopentaPAH) are metabolically activated to mutagenic and carcinogenic species via cyclopenta epoxidation[1], no DNA adduct of a cyclopenta epoxide has been characterized[2]. We therefore wished to develop synthetic routes to adducts anticipated to result from attack of cyclopenta epoxides at N^6 of adenine and N^2 of guanine. Pursuant to this objective, we report the synthesis of the diastereomeric pairs N^6-[1-(trans-2-hydroxyacenaphthenyl)] and N^6-[1-(cis-2-hydroxyacenaphthenyl)] adenosine (**1a,b** and **1c,d** respectively) and N^6-[1-(trans-2-hydroxyacenaphthenyl)] adenosine (**2a,b**). The key reaction in the synthetic scheme was the condensation of enantiomeric 1-amino-2-hydroxy cyclopentaPAH mixtures with 6-chloropurine-9-β-D-ribofuranose to give diastereomeric mixtures readily separated and purified by reversed phase HPLC. Since the trans amine alcohols (3t,4t) were of paramount interest as synthons, Scheme 1 was adopted. Reaction

Scheme 1



of the cyclopenta 1H-2-oxo compounds[3,4] with i-amylnitrite yielded 1-oxime-2-oxo derivatives **5** and **6**, as described in the literature[3]; however, the work-up was modified to obtain pure oxime ketones by preparative TLC on silica developed with chloroform. The oxime functionality of **5** and **6** was reduced selectively[5] by partial hydrogenation over PtO_2 to give amine ketones **7** and **8**, obtained as hydrochlorides after filtration to remove PtO_2, evaporation of filtrate and trituration with methylene chloride. The hydrochlorides were dissolved in methanol (0.1 M solution) and an equal weight of $NaBH_4$ added with stirring at room temperature. The reduction was followed by UV-vis spectroscopy, monitoring the development of the PAH chromophores (~3/4 h). After stirring the acidified reaction (1:1 H_2O:HOAc) for 1 h, the solvent was evaporated and the residue dissolved in water,

neutralized with aqueous Na_2CO_3 and the amine alcohol extracted into chloroform. UV-vis spectra, mass spectra and yields for 3-8 are given in Table 1. Minor amounts of cis amine alcohols were identified in the $NaBH_4$ reduction mixtures by comparison of ^1H NMR spectra with the spectra of pure cis isomers 3c, 4c from complete hydrogenation[5] of 5 and 6 over PtO_2. The predominance of 3t and 4t in the $NaBH_4$ reduction (3t:3c, 3:1, 4t:4c, 5:1) was determined by comparing the integrated resonances of ethano protons H_1 and H_2.

Table 1. Characterization of intermediate compounds

Compound	% Yield	UV-vis (methanol) $\lambda_{max}(\epsilon x10^{+3})$ nm	EI Mass spectrum (70 eV), m/z (rel. intensity)
Acenaphthene			
1-oxime-2-one (5)	70	270(12.20)	197(100), 181(10), 168(12), 153(50)
1-amine-2-one HCl (7)	78	284(5.54)	
trans 1-amine-2-ol (3t)	100[a,6]	286(5.44)	185(70), 168(100), 167(72), 156(40)
cis 1-amine-2-ol (3c)	50[b,6]		
Aceanthrene			
1-oxime-2-one (6)	55	403(7.18), 383(6.44) 362(6.19), 252(92.55)	247(65), 230(100), 202(65)
1-amine-2-one HCl (8)	69	383(4.74), 366(4.74), 346(4.35), 2.54(47.83), 245(60.47)	
trans 1-amine-2-ol(4t)	98[a,6]	385(4.29), 366(4.50), 348(3.33), 322(1.99), 255(70.63)	235(100), 217(74), 206(67), 189(63), 178(48)
cis 1-amine-2-ol (4c)	50[b,6]		

[a]Yield, $NaBH_4$ reduction. [b]Yield, H_2/PtO_2 reduction.

The final condensation step was accomplished by refluxing 6-chloropurine ribofuranoside with a 3-fold molar excess of amine alcohol for 25 h in ethanol containing triethylamine[7] Typical reaction scales were: 150 mg 3t and 73 mg 6-chloropurine ribofuranoside in 15 mL ethanol with 5-6 drops of triethylamine; 30 mg 4t and 12 mg 6-chloropurine ribofuranoside in 5 mL ethanol with 2 drops of triethylamine. After evaporation of solvent, the solid residue was dissolved in methanol and an initial separation performed by HPLC on a 9.4 x 250 mm Zorbax column. Collected peaks were screened by UV-vis for the PAH chromophores and the presence of modified nucleosides confirmed by collisionally activated decomposition-tandem mass spectrometry using a fast atom bombardment source (FAB CAD MS/MS). Two major products (**1a,b**; **2a,b**) were identified from the condensation of each enantiomeric

trans amine alcohol mixture and purified by additional HPLC. Yields, product ratios and separation parameters are given in Table 2. Formation of the adenosine derivatives does not involve the PAH periphery, therefore, condensation products of the trans enantiomers will yield corresponding sets of trans diasteriomers, and structural assignments 1a,b and

Table 2. HPLC Separation of N^6-Modified Adenosine

Initial Separation

Cpd	Solvent Program (%MeOH in H_2O at 4 mL/min)	Diastereomer Retn. Time (min)(Rel. Ratio)	
3t	5 min @ 40%; 40→55% in 10 min; 55→85% in 10 min	13(1)	15(1.2)
3c	5 min @ 40%; 40→55% in 10 min; 55→85% in 10 min	11.5(1)	12.5(1.1)
4t	40→80% in 40 min	16.5(1)	18.0(2)

Final Purification

Cpd	Solvent Program (% MeOH in H_2O at 4 mL/min	Diastereomer Retn. Time (min)	Total Yield (%)[a]
3t	40%, isocratic	13.5, 15.5	7
3c	40%, isocratic	11.5, 13	2
4t	40→80% in 40 min	16.5, 18.0	7

[a]Yield based on 6-chloropurine ribofuranose

2a,b have been made accordingly. Condensation of 3c gave two major products with consistent with formation of the cis diastereomeric pair 1c,d. Resolution of all four expected diastereomers was demonstrated in the condensation of a 1:1 3t:3c mixture (from LAH reduction of 5). Compounds 1a - d have identical UV-vis spectra and fragmentation patterns by FAB CAD MS/MS. UV-VIS (methanol): $\lambda_{max}(\epsilon \times 10^{+3})$ nm, 275(12.69) nm, FAB CAD MS/MS: m/z 436$(M+H)^+$, 304(M-ribosyl), 169(M+H-Ado), 133$(ribosyl)^+$. The low-field region of the ^1H NMR spectra of trans diastereomers 1a,b is distinctly different from the low field region of cis diastereomers 1c,d (Fig. 1a,b).

The trans diastereomers 2a,b have identical UV-vis spectra and fragmentation patterns by FAB CAD MS/MS. UV-vis (methanol): $\lambda_{max}(\epsilon \times 10^{+3})$ nm, 387(3.82) nm, 270(9.82), 255(50.0) nm, FAB CAD MS/MS: m/z 486$(M+H)^+$, 354(M+H-ribosyl), 336(M+H-ribosyl-H_2O), 268 (Ado+H), 219(M+H-Ado). The ^1H NMR spectra of 2a,b (Fig. 2) are also identical.

The condensation of 6-chloropurine riboside with alkyl amines or p-substituted anilines is more efficient (yields: 20 - 80%[8,9]) than with the polycyclic aromatic amine alcohols reported here. Nevertheless, this report provides a route to a class of modified nucleosides in quantity that would heretofore have been available only in microgram amounts from modification of polyribonucleic acids.

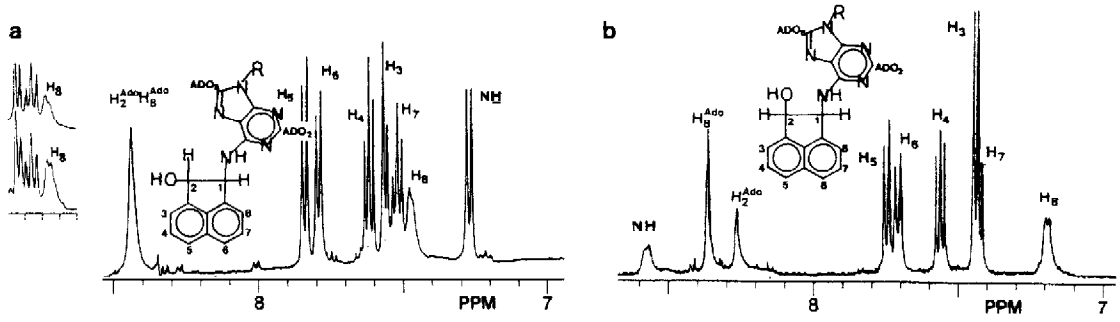


Fig. 1. ^{1}H NMR (500MHz, DMSO-d_6, room temp.) of adenosine adducts from 3t (a) and 3c (b). H_3 was distinguished from H_8 by the proximity of H_8 to the effects of nucleoside; remaining assignments followed from COSY studies. The cis diastereomers have identical traces, while a slight change signal width is apparent for H_8 of the trans pair (inset).

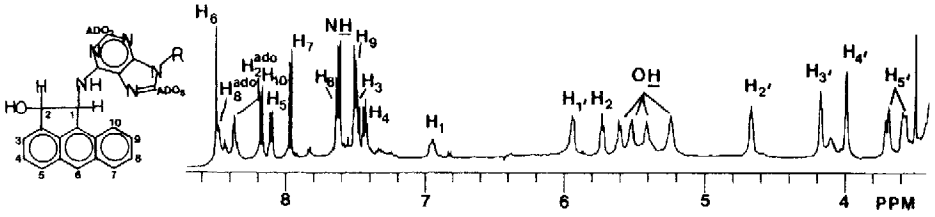


Fig. 2. ^{1}H NMR (500MHz, DMSO-d_6, room temp.) of adenosine adducts from 4t. Assignments of proton resonances made from COSY study.

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6. Chemical shifts of ethano protons (400 MHz, DMSO-d_6), 3t: 4.95(br. triplet H_1), 4.24(br. doublet H_2) ppm; 3c; 5.08(d, H_1), 4.50(d, H_2), ppm, $J_{1,2}$=5.2Hz; 4t: 5.34 (d, H_1), 5.13(d, H_2) ppm, $J_{1,2}$=6.1Hz; 4c: 5.79(d, H_1), 5.67(d, H_2) ppm, $J_{1,2}$=5.8Hz.

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