SYNTHESIS OF A NON-SACCHARIDAL ANALOG OF PUROMYCIN[1].

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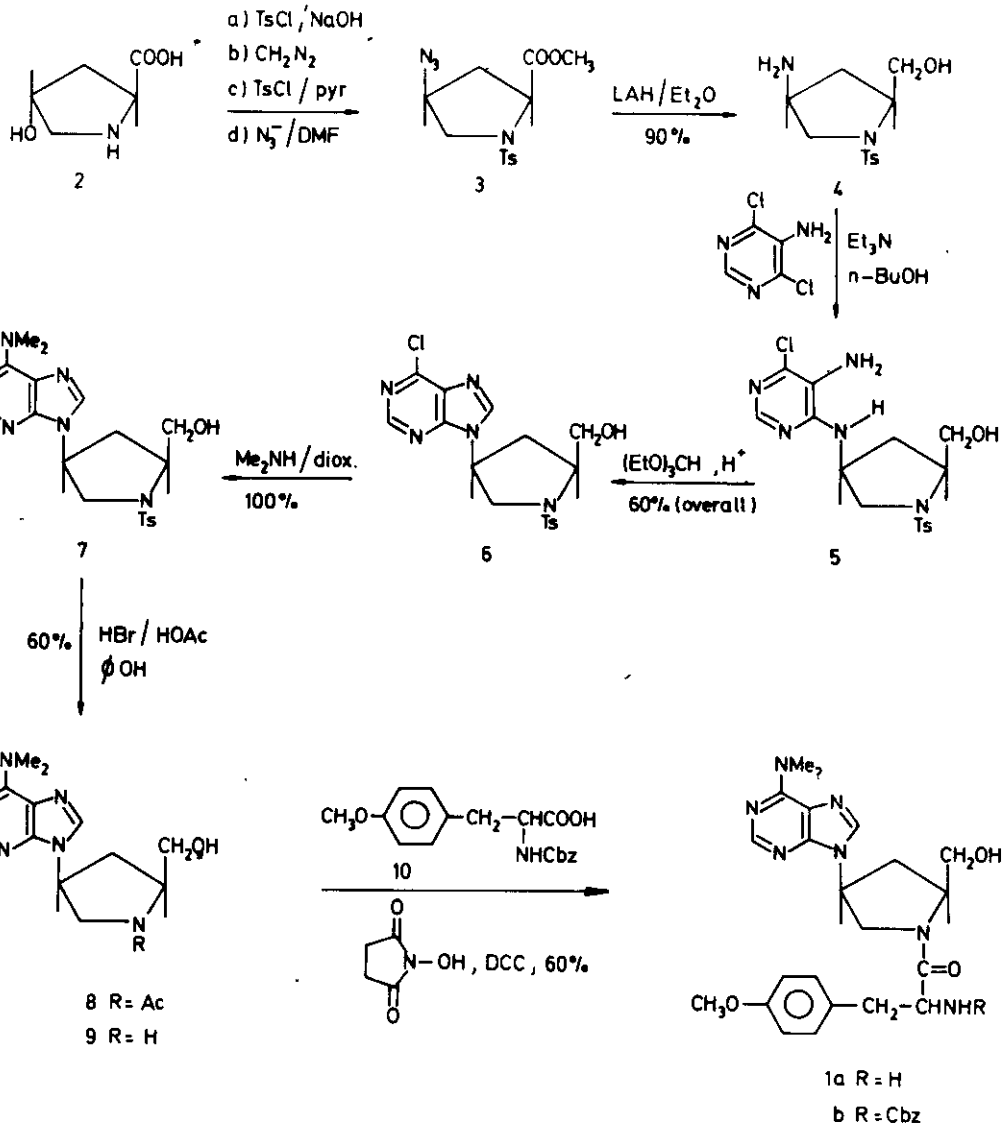
The synthesis of (2S,4S)-N-(p-methoxyphenyl-L-alanyl)-2-hydroxymethyl-4-[N_9-6-(dimethylamino)purinyl]pyrrolidine (1), a non-saccharidal analog of puromycin, is described.

Several non-saccharidal nucleoside analogs in which the purine and pyrimidine bases are attached to α-amino acids have been recently reported from this laboratory.[3,4] Particular interest resides in these systems in view of their analogy to nucleo-peptide models.[5] Amongst the natural α-amino acids L-proline possesses structural features which make it uniquely suitable as a substitute for the glycosidic moiety of the nucleoside analogs.[6] This communication describes the synthesis of the novel puromycin analog 1a.

The commercially available optically active (-)-4-hydroxy-L-proline (2) was visualized as a convenient starting material for the synthesis of 1. N-Tosylation of 2, followed by the sequence esterification, O-tosylation and reaction with azide anion led, via stereospecific substitution (SN_2) of the O-tosyl group, to azide 3.[7] Reduction of the azide with $LiAlH_4$ yielded the versatile intermediate 4, mp 119-120° (90%), in which the amino group is capable of elaboration into a desired purine or a pyrimidine derivative.[6] Conversion of 4 into the 6-chloropurine derivative 6 [mp 174-176°; uv (C_2H_5OH) 230 nm (ε 16,000), 265(10,500); nmr($CDCl_3$), δ 8.42, 8.69 (2H, 2 x s, purine protons[8])] was carried out in two


a) TsCl / NaOH
b) CH_2N_2
c) TsCl / pyr
d) N_3^- / DMF
LAH / Et_2O
90 %
Et_3N
n-BuOH
Me_2NH / diox.
100 %
$(EtO)_3CH$, H^+
60 % (overall)
60 %
HBr / HOAc
ϕOH
10
N-OH , DCC , 60 %
2
3
4
5
6
7
8 R = Ac
9 R = H
1a R = H
b R = Cbz

steps, namely, via its condensation with 5-amino-4,6-dichloropyrimidine and subsequent cyclization of the resulting pyrrolidine derivative 5 by heating it with ethyl orthoformate (pure) in the presence of catalytic quantities of HCl.

Treatment of 6 with dimethylamine in dioxane yielded, in quantitative yield, the corresponding 6-dimethylamino derivative 7 [mp 75-85°; uv (C_2H_5OH) 218 nm (ε 19,500), 276 (ε 16,000); nmr ($CDCl_3$) δ 3.50 (6H-s, $N(CH_3)_2$), 7.81, 8.26 (2H, 2 x s, purine protons[8])]. Attempted detosylation of 7 (HBr/HOAc, PhOH) gave, in addition to the expected free amine 9, the corresponding N-acetylated product 8 [ir 1630 cm^{-1} ($NCOCH_3$); nmr ($CDCl_3$) δ 2.11 (3H-broad singlet $NCOCH_3$)]. The mixture of 8 and 9 could be smoothly converted to 9 by treatment with KOH and methanol.

The coupling of 9 with protected p-methoxyphenylalanine 10 was most satisfactorily achieved by the use of DCC and N-hydroxysuccinimide.[9] The latter procedure is known to involve little or no racemization during peptide bond formation and has been successfully applied to the synthesis of certain puromycin analogs.[9] When the resulting dipeptide 1b was subjected to hydrogenolysis (H_2/Pd, HOAc) the benzyloxycarbonyl (Cbz) group was readily removed to yield the puromycin analog 1a as a hygroscopic solid, mp 72-75°, [ir (KBr) 1630 cm^{-1} (N-C=O), 1590 (purine); uv (C_2H_5OH) 277 nm (ε 18,500)].

The nmr spectrum of 1a in $CDCl_3$ (30°) exhibits two analogous sets of partly superimposed signals. When, however, the spectrum was run at 100° (d_6-DMSO), several of the bands sharpened and the double sets of signals disappeared to result in a spectral pattern typical of a single species (Table I).

TABLE I

Temp.	$N(CH_3)_2$	$p\text{-}MeOC_6H_4$ (aromatic)	H_2, H_8 (purine)
30^0 ($CDCl_3$)	3.49 (s)[b]	6.73, 6.81; 7.05, 7.13	7.61; 8.26
30^0 ($CDCl_3$)	3.49 (s)[b]	a 6.91; 7.09, 7.17	7.81; 8.24
100^0 (d_6-DMSO)	3.44 (s)	6.76, 6.85; 7.09, 7.18	8.03; 8.21

a. Falls under signal at δ 6.81; b. broadened singlet.

These results can be rationalized in terms of the existence at 30^0, of two discrete amide configurational isomers (I and II) involving a restricted rotation about the amide bond. This type of isomerization has been well documented.[10] The possibility that steric hinderance to rotation of the purine moiety (about C-N bond) may account for the two isomers can be excluded from the fact that while compounds like 6 and 7 - in which steric hinderance would be appreciable - do not exhibit such isomerism, the nmr spectrum of the N-acetyl derivative 8 is consistent with existence of two 'frozen' rotamers. The rotational barrier is, however, insufficient to maintain the integrity of the two species at higher temperatures and the observed spectrum at 100^0 is that of a single substance. From the integrals of the aromatic and purine protons the ratio of I:II has been determined as 2:1.[11] Biological investigations on the puromycin analog 1a are in progress and their results will be presented elsewhere.

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11 While a tentative conformational assignment of the rotamers has been made, these results will be discussed in detail separately.

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