Communications to the Editor

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NEW DIMERIC ANALOGS OF 2-AMINODIPYRIDO[1,2-a:3',2'-d]IMIDAZOLE: SYNTHESIS AND INTERACTION WITH DNA

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New dimeric analogs of intercalative 2-aminodipyrido-[1,2-a:3',2'-d]imidazole (Glu-P-2), a potent muta-carcinogen isolated from a pyrolysate of L-glutamic acid, were synthesized. The high affinity of these compounds to DNA was demonstrated by fluorescence spectroscopic studies and by their effects in raising the melting temperature (T_M) of DNA.

KEYWORDS —— intercalation; 2-aminodipyrido[1,2-a:3',2'-d]imidazole; DNA; melting temperature of DNA; dimeric intercalator

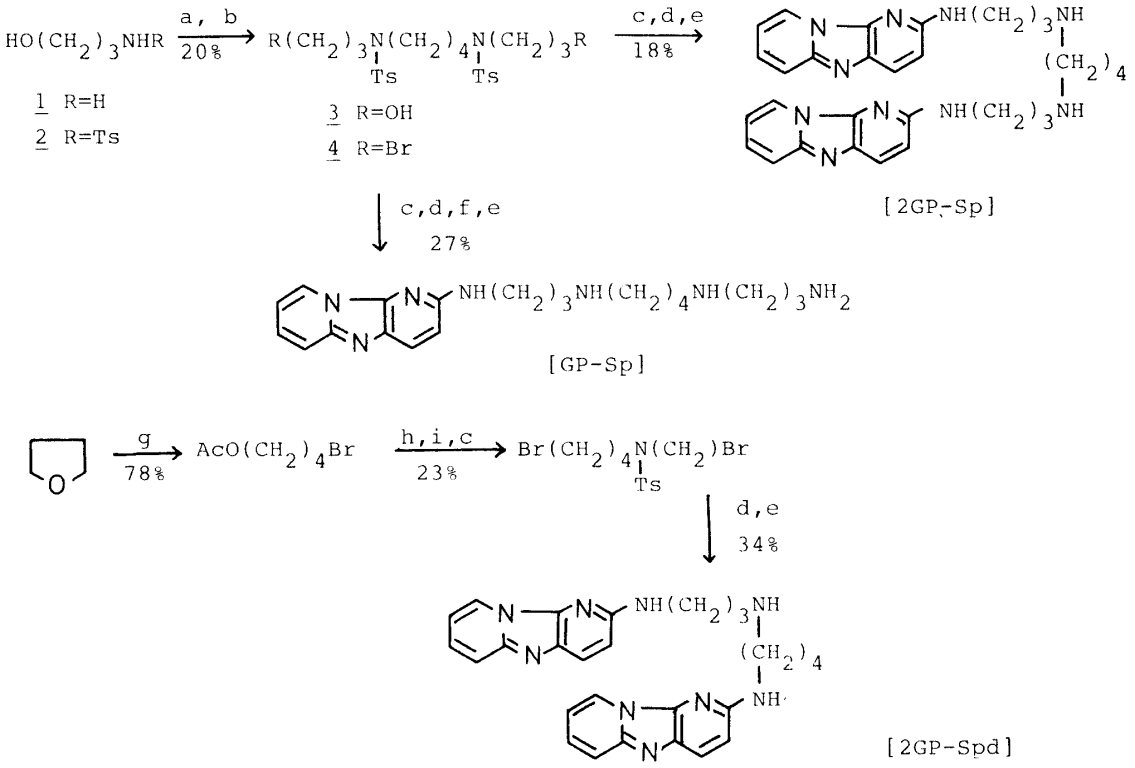
Intercalation is one of the important modes of interaction of muta-carcinogenic or antitumor agents with DNA. Recently, some dimeric analogs of intercalators such as ethidium bromide[1] were shown to possess an increased affinity toward DNA. On the other hand, potent muta-carcinogens in cooked foods such as 3-aminopyrido[4,3-b]indoles (Trp-P's)[2] and 2-aminodipyrido[1,2-a:3',2'-d]imidazoles (Glu-P's)[3] are now attracting attention. These amines bind covalently to DNA after metabolic activation.[4] In addition, the authors showed that Glu-P's intercalate to DNA with the comparable affinity of ethidium bromide,[5] and this ability is important for the covalent binding of the amine to DNA.[4] In this communication, we describe the synthesis of potent intercalative analogs of 2-aminodipyrido[1,2-a:3',2'-d]imidazole (Glu-P-2). We chose spermine or spermidine as a covalent connector of the parent intercalator, Glu-P-2, because these polyamines are known to possess high affinity toward DNA.[6]

Synthesis:

Glu-P-2 was prepared as described previously.[7] Bis-Glu-P-2-spermine (2GP-Sp), Glu-P-2-spermine (GP-Sp) and bis-Glu-P-2-spermidine (2GP-Spd) were prepared by the routes shown in Scheme 1. N-Tosyl-Glu-P-2 was prepared by tosylation of Glu-P-2 with TsCl in pyridine (y:81%, mp. 236-237°C). The terminal synthetic step for the preparation was the nucleophilic substitution of the appropriate alkyl bromide by the tosylamide anion produced by use of NaH. The tosyl group protects the further dialkylations and were removed by acid hydrolysis. 2GP-Sp, GP-Sp and 2GP-Spd were obtained as HBr salts, purified by

recrystalization from EtOH to give needles. The structures were deduced from their ^{1}H-NMR (obtained in D_2O with a reference compound of TSP-Na or in CD_3OD with a reference of TMS), UV, and elemental analysis (data are shown below).

Scheme 1



a: TsCl, K_2CO_3, DMF, 125°C; b: $Br(CH_2)_4Br$, DMF, 120°C; c: $SOBr_2$, $CHCl_3$, reflux; d: N-tosyl-Glu-P-2, NaH, DMF; e: 47%HBr, reflux; f: NH_3, EtOH, 110°C; g: $ZnCl_2$, AcBr, 120°C; h: $TsNH(CH_2)_3OH$, NaH, DMF, 100°C; i: HCl, MeOH, reflux.

2GP-Sp-5HBr: mp.262°C; Anal. calcd. C: 38.26, H: 4.35, N: 14.88; found C: 38.55, H: 4.47, N: 14.58; ^{1}H-NMR (D_2O, TSP-Na): 1.64-2.28 (m,8H), 3.00-3.36 (m,8H), 3.54 (t,J=8Hz,2H), 6.86 (d,J=9Hz,2H), 7.45 (t,J=8Hz,2H), 7.68-8.08 (m,6H), 8.82 (d, J=7Hz,2H); UV (H_2O, λmax): 258, 303, 317, 377 nm.

GP-Sp-4.5HBr: mp.267°C; Anal. calcd. C: 32.74, H: 4.90, N: 13.36; found C: 32.72, H: 4.91, N: 13.27; ^{1}H-NMR (D_2O, TSP-Na): 1.68-1.88 (m,4H), 1.96-2.28 (m,4H), 3.00-3.32 (m,10H), 3.62 (t,J=8Hz,2H), 6.97 (d,J=9Hz,1H), 7.49 (t,J=8Hz,1H), 7.88-8.08 (m,3H), 8.95 (d,J=8Hz,1H); UV (H_2O, λmax): 257, 303, 312, 370 nm.

2GP-Spd-2HBr-H_2O: mp.275°C; Anal. calcd. C: 39.46, H: 4.26, N: 15.34; found C: 39.84, H: 3.92, N: 14.94; ^{1}H-NMR (CD_3OD, TMS): 1.76-2.00 (m,4H), 2.04-2.30 (m,4H), 3.04-3.28 (m,4H), 3.48-3.76 (m,4H), 7.03 (d,J=9Hz,1H), 7.05 (d,J=9Hz,1H), 7.65 (t,J=8Hz,2H), 7.84-8.20 (m,6H), 9.06 (d,J=7Hz,1H), 9.15 (d,J=7Hz,1H); UV (H_2O, λmax): 254, 303, 312, 370 nm.

Affinity toward calf thymus DNA:

The C_{50} value[8] for ethidium displacement, the micromolar concentration of added drug necessary to displace 50% of the DNA-bound ethidium, was measured in order to deduce the drug-DNA affinities (Table 1). Calf thymus DNA (Sigma,

Table 1. C_{50} values[a] for ethidium displacement

drug	C_{50} (μ mole)	drug	C_{50} (μ mole)
EtBr	1.25	2GP-Sp	0.11
Glu-P-2	1100	GP-Sp	0.13
spermine	0.23	2GP-Spd	6.5
spermidine	1.00	spermine + Glu-P-2[b]	0.24

a) The C_{50} value is defined as the micromolar concentration of added drug necessary to displace 50% of the DNA-bound ethidium (calf thymus DNA: 0.98 μM, EtBr: 1.25 μM, in 1mM phosphate buffer, pH 7.3). It is measured by the fluorescence intensity of DNA-bound ethidium (excited at 546 nm, emitted at 655 nm).

b) Glu-P-2 was added twice as molar amount of spermine.

Type 1) was purified by ultra-centrifugation in a buffer (14×10^4G for 40 min). The affinity of monomeric Glu-P-2 was very weak compared to EtBr. Spermine possesses rather high affinity. Though addition of Glu-P-2 to spermine caused no effect on the ethidium displacement by spermine alone, dimeric 2GP-Sp as well as monomeric GP-Sp showed an enhanced affinity to DNA. Covalent connection of Glu-P-2 to spermidine did not cause such an effect. The affinities of drugs decrease in the order of; 2GP-Sp > GP-Sp > spermine > spermidine > EtBr > 2GP-Spd ≫ Glu-P-2.

Effects on melting temperature (T_M):

The ability of dimeric analogs of Glu-P-2 to protect calf thymus DNA from heat denaturation was measured by UV spectrophotometry,[9] i.e., by the increase in the optical density at 260 nm upon heat denaturation (Table 2). The melting

Table 2. Melting temperature (T_M) of calf thymus DNA[a]

drug	T_M (°C)	drug	T_M (°C)
none	70.0	2GP-Sp	83.5
EtBr	76.1	GP-Sp	82.5
Glu-P-2	71.5	2GP-Spd	71.8
spermine	78.5	spermine + Glu-P-2[b]	78.6

a) In 0.1 SSC (0.015 M NaCl, 0.0015 M Na_3-citrate). Calf thymus DNA: 4.29×10^{-2} mM P. Drug: 4.29×10^{-3} mM P.

b) Spermine (4.29×10^{-3} mM) and Glu-P-2 (8.58×10^{-3} mM).

temperature (T_M) of native calf thymus DNA measured in 0.1 SSC was 70.0°C, which was in good conformance with the reported one.[9] The drugs were added in the amount of one molecule per 10 nucleotides in the DNA. Glu-P-2 and, surprisingly, 2GP-Spd showed almost no effect on T_M. Spermine rather strongly increased the T_M as reported,[6] and addition of Glu-P-2 did not increase the effect of spremine

alone. 2GP-Sp and GP-Sp unexpectedly raised the T_M markedly, exceeding the effect of EtBr comparable to that of bis-ethidium analogs.[1] When 2GP-Sp was added in amount of one molecule per 2 nucleotides in the DNA, the T_M was raised to 91.5°C. The effect decrease in the order of: 2GP-Sp > GP-Sp ≫ spermine > EtBr ≫ 2GP-Spd > Glu-P-2.

Conclusion:

Glu-P-2 covalently connected to spermine, 2GP-Sp and GP-Sp, had a high affinity with calf thymus DNA and, unexpectedly, strongly increased the T_M of the DNA. These effects may be attributable to the potentiating effect of the intercalative binding of Glu-P-2 to DNA and the electrostatic binding of spermine to DNA, produced by the covalent connection of these drugs. Addition of Glu-P-2 to spermine did not increase the effect of spermine alone. Another dimeric analog, 2GP-Spd, showed negligible effects. The biological activities such as antitumor activity of these drugs are under investigation.

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