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SYNTHESIS OF POTENTIAL INHIBITORS OF TRANSMETHYLASES

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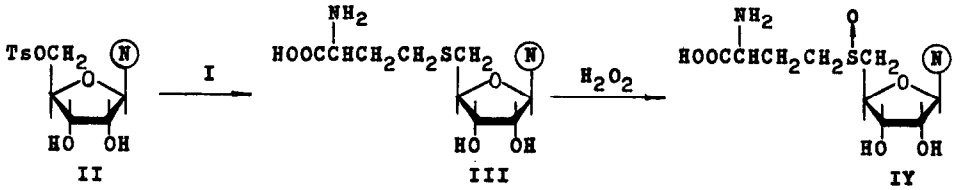
S-Adenosyl-L-homocysteine (SAH, IIIa) is a product of all biological transmethylations with (-)-S-adenosyl-L-methionine ("active methionine") as methyl donor. SAH has been found to be an effective inhibitor in some of such reactions[1-3]. Hence it is clear why different structural analogs of SAH[2,4-6] are considered as potential inhibitors or regulators of this important biological process.

The synthesis of SAH (IIIa) has been reported previously via its 2',3'-O-isopropylidene derivative[7]. Prolonged acid hydrolysis of the latter led to SAH. But this compound is known to be unstable in acid solutions and readily hydrolyzed to adenine and S-ribosyl-L-homocysteine.

We report here a facile method for the direct preparation of SAH (IIIa) and its nucleoside analogs--S-nucleosyl-L-homocysteines (SNH-s, b-c) from the 2',3'-O-unprotected 5'-O-tosylnucleoside (II) and the disodium salt of L-homocysteine (I) in liquid ammonia. Similar results were obtained with the compound (I) prepared by reduction of L-homocystine or L-methionine. By this method S-adenosyl- (SAH, IIIa), S-inosyl- (SIH, IIIb), S-guanosyl- (SGH, IIIc), S-uridyl- (SUH, IIId) and S-cytidyl- (SCH, IIIe)-L-homocysteines have been synthesized. In this series SIH (IIIb) had been obtained by enzymatic deamination of SAH (IIIa)[4], but not fully characterized. The uridyl and guanosyl analogs were prepared as racemic DL-compounds[5].

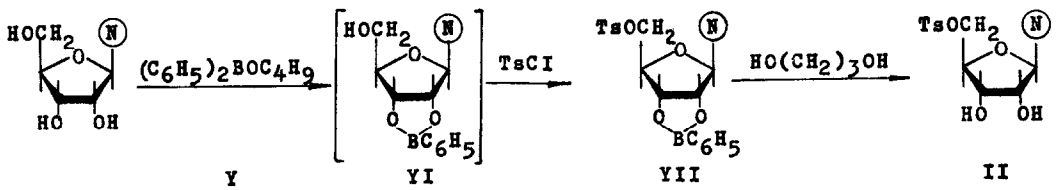
Oxidation of the SNH-s (IIIa-e) by hydrogen peroxide gave their sulfoxides (SNHO-s, IVa-e)--structural analogs of S-adenosyl-L-methionine.



N: IIa, IIIa, IVa = 9-adeninyl
IIb, IIIb, IVb = 9-hypoxanthinyl
IIc, IIIc, IVc = 9-guaninyl

N: IId, IIId, IVd = 1-uracyl
IIe, IIIe, IVe = 1-cytosinyl

5'-O-Tosyladenosine (IIa) and 5'-O-tosyluridine (IId) were obtained by the reaction of the 2',3'-O-phenylboronate esters of adenosine and uridine with p-toluenesulphonyl chloride, followed by removal of the phenylboronate protecting group by treatment with propan-1,3-diol[8]. Attempts to prepare 5'-O-tosylinosine (IIb) and 5'-O-tosylguanosine (IIc) by this method in good yield were unsuccessful because of unstability of the 2',3'-O-phenylboronate esters of inosine (VIb) and guanosine (VIc). Therefore a modified method, excluding the formation of water during the reaction, has been worked out. The esters (VIb) and (VIc) were obtained by refluxing a mixture of the nucleoside with the isobutyl ester of diphenylboronic acid (V) in anhydrous pyridine. Then without isolation from the solutions the compounds (VIb) and (VIc) were treated by p-toluenesulphonyl chloride and converted into the tosylates (VIIb) and (VIIc). The use of the ester (V) instead of phenylboronic acid prevented the formation of water, which appears to cause hydrolysis of the unstable 2',3'-O-phenylboronate esters (VIb) and (VIc).



N: VIb, VIIb, IIb = 9-hypoxanthinyl
VIc, VIIc, IIc = 9-guaninyl

In this way, the tosylate (VIIb) was obtained in 66% yield. Treatment of VIIb with propan-1,3-diol gave 5'-O-tosylinosine (IIb) (93%). Anal. Calcd. for $C_{17}H_{18}N_4O_7S$: C, 48.34; H, 4.36; N, 13.26. Found: C, 48.27; H, 4.28; N, 12.88%.

UV-Spectrum (methanol): λ_{max} = 249 (3.99), 224 (4.17) nm (log ε). Similarly, the tosylates of guanosine (VIIc) and (IIc) were isolated in 59% and 93% yield, respectively. Anal. Calcd. for $C_{17}H_{19}N_5O_6S$: C, 46.68; H, 4.38. Found: C, 47.12; H, 4.45. UV-Spectrum (methanol): λ_{max} = 253 (4.01), 224 (4.15) nm (log ε).

The synthesis of 5'-O-tosylcytidine (IIe) started from 4-exo-N-acetylcytidine[9] to avoid the formation of the isomeric 4-exo-N-tosylate[10]. The 5'-O-tosylate of 4-exo-N-acetylcytidine was obtained by the method[8] in 71% yield, calculating on starting acetylcytidine. Anal. Calcd. for $C_{18}H_{21}N_3O_8S$: C, 49.19; H, 4.82; N, 9.56. Found C, 49.48; H, 4.81; N, 9.56%. UV-Spectrum (methanol): λ_{max} = 297 (3.91), 248 (4.19), 222 (4.28) nm (log ε). After removal of the acetyl protecting group by action of methanolic ammonia 5'-O-tosylcytidine (IIe) was obtained in 81% yield. Anal. Calcd. for $C_{16}H_{19}N_3O_7S$: C, 48.37; H, 4.82; N, 10.57. Found: C, 48.67; H, 4.90; N, 9.81 . UV-Spectrum (methanol): λ_{max} = 271 (3.58), 224 (4.22) nm (log ε).

The following general procedures were used for the preparation of SNH (III) and its sulfoxide (SNHO, IV). To a solution of the disodium salt of L-homocysteine (I) (2 mmol) (from L-homocystine or L-methionine and sodium) in liquid ammonia (100 ml) was added 5'-O-tosylnucleoside (II) (4 mmol). The reaction mixture was stirred for two hours, and then the solvent was allowed to evaporate. The residue was dissolved in water (50 ml) and passed through a column of KU-2 ion-exchange resin (NH_4^+-form) to remove sodium ions. SNH (III) was eluted by water. The eluate was concentrated in vacuo and passed through a column of DEAE-cellulose (OH^--form) to remove coloured impurities. The aqueous eluate was evaporated to dryness in vacuo. Crystallization of the residue from aqueous ethanol gave an analytical sample of SNH (II).

SNH (III) (1 mmol) was oxidised with hydrogen peroxide (3 mmol) at 20° and pH 6.5 for 16 hours. The excess of H_2O_2 was decomposed by addition of 5% Pd/C. The aqueous solution was evaporated to dryness in vacuo. The sulfoxide (IV) was crystallized from aqueous ethanol.

The purity and structures of all compounds obtained were established by paper and thin layer chromatography, IR- and UV-spectrophotometry and elemental

analysis. The infrared spectra of the SNHO-s (IVa-e) had bands at 1010-1035 cm^{-1}, which were assigned to the $-C-\overset{O}{\overset{\|}{S}}-C$ group.

Yield, $[\alpha]_D^{30}$, M.p., and UV-spectral data of SNH(III) and SNHO(IV) are presented in the Table.

Table

Compound	Yield,%	M.p., °C(decomp.) (aqueous ethanol)	$[\alpha]_D^{30}$ in ° (c 0.6,water)	UV-spectrum (water) λ max	log ε
SAH (IIIa)	56	208-210	0[x]	260	4.17
SAHO (IVa)	72	-[xx]	+26.7	260	4.05
SIH (IIIb)	41	186-188	+13.3	249	4.09
SIHO (IVb)	67	-	+36.3	249	4.05
SGH (IIIc)	30	234-237	-20.0	252	4.10
SGHO (IVc)	71	-	+ 6.7	252	4.06
SUH (IIId)	55	210-212	+33.3	260	3.90
SUHO (IVd)	53	-	+50.0	260	3.88
SCH (IIIe)	43	184-186	+56.7	270	3.79
SCHO (IVe)	58	-	+56.7	270	3.50

[x] +38° (c1, 0.2N HCl)

[xx] SNHO-s melted with decomp. at above 100° over a large temperature range.

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