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SYNTHESIS OF9-[5′-DEOXY-5′-(ALKYLTHIO)-β-D-XYLOFURANOSYL] ADENINES AND THEIR ANTIVIRAL ACTIVITY*

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9-[5′-Deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine (12), 9-[5′-deoxy-5′-(isopropylthio) (13), 9-[5′-deoxy- 5′-(isobutylthio) (14) and 9-[5′-deoxy-5′-n-heptylthio)-β-D-xylofuranosyl]adenines(15) have been synthesized and evaluated for antiviral activity. However, none of these exhibited any promising activity against Ranikhet disease virus (RDV).

S-Adenosyl-L-methionine[1] (AdoMet) a ubiquitous enzyme that occurs both in normal and malignant tissues acts as methyl group donor in transmethylation reactions[2] and as a propylamine donor in polyamine synthesis[3,4]. In these transformations AdoMet is converted into S-adenosyl-L-homocysteine (SAH) (**1**) and 5′-deoxy-5′-(methylthio)adenosine (MTA) (**2**). Both of these metabolites are known to inhibit the transmethylation reactions of AdoMet *in vitro*[5-7]. Further **2** is known to be involved in several regulatory processes[8]. AdoMet analogues such as sinefungin(**3**) is reported to inhibit methyl-transferases[9]. 5′-Deoxy-5′-(isobutylthio)adenosine (SIBA) (**4**) strongly inhibited oncogenic transformation of chick embryo fibroblast[10], polyoma virus replication in mouse embryo fibroblast[11], replication of Herpes virus[12], of mouse mammary tumor virus[13] and the multiplication of the malaria parasite (*Plasmodium falcipanum*) in erythrocyte cultures[14].

Recently 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl] adenine (**12**), the first naturally occurring analogue of methylthio adenosine (MTA) (*2*), was isolated from the marine nudibranch mollusc *Doris verrucosa*[15]. Although couple of synthetic procedures for **12** have been reported[16,17], but there has been no attempt to evaluate its biological activity.

In view of this, it was thought worthwhile not only to develop an efficient synthesis of **12** and some of its analogues but also to evaluate them for their biological activity.

In the present communication we report an alternate synthesis of 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl] adenine (**12**) as well as the synthesis of 9-[5′-deoxy-5′-(isopropylthio)-β-D-xylofuranosyl]adenine (**13**), 9-[5′-deoxy-5′-(isobutylthio)-β-D-xylofuranosyl] adenine (**14**), and 9-[5′-deoxy-5′-(n-heptylthio)-β-D-xylofuranosyl] adenine (**15**) and their antiviral activity against Ranikhet disease virus (RDV).

Condensation of adenine (**5**) (Scheme-1) with 1,2,3,5-tetra-O-acetyl-D-xylofuranose (**6**) in the presence of stannic chloride afforded the blocked xyloside[18] (**7**) in very good yield. The β-configuration of xylose moeity at position 9 of adenine was established with the help of PMR spectral data. Deblocking of the nucleoside (**7**) with methanolic ammonia afforded 9-β-D-xylofuranosyl adenine[18] (**8**). Selective tosylation of hydroxy function at position C-5with pyridine and *p*-toluenesulfonyl chloride at 0-5° yielded 9-[5′-deoxy-5′-(tosyl)-β-D-xylofuranosyl] adenine (**9**). Acetylation of **9** with acetic anhydride/pyridine afforded 9-[5′-deoxy-5′-(tosyl)-2′,3′-di-O-acetyl-β-D-xylofuranosyl]adenine (**10**). Treatment of **10** with methylmercaptan in DMF gave 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine (**11**).

Removal of acetyl protecting groups from **11** with methanolic ammonia finally furnished 9-[5′-deoxy-5′-(methylthio)-β-D-xylofuranosyl]adenine(**12**). The PMR, MS, and UV spectral data of the compound **12** were almost identical with reported data[15].

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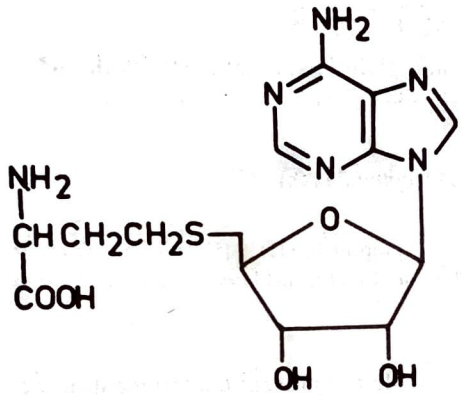

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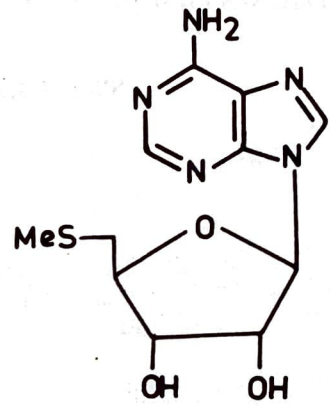

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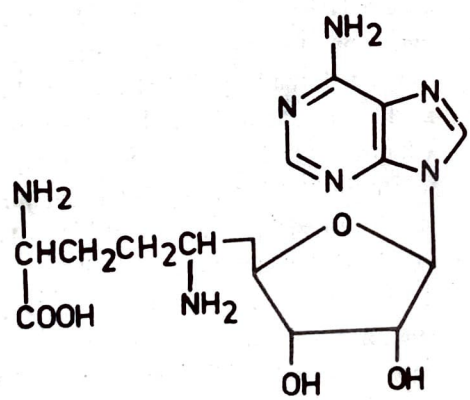

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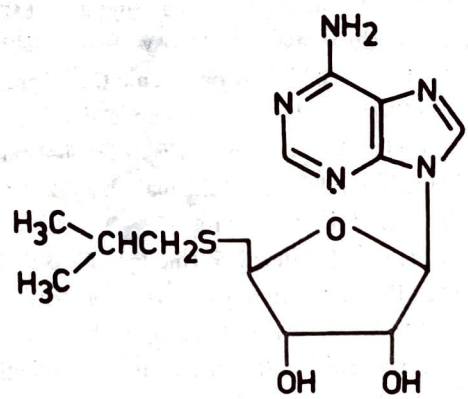

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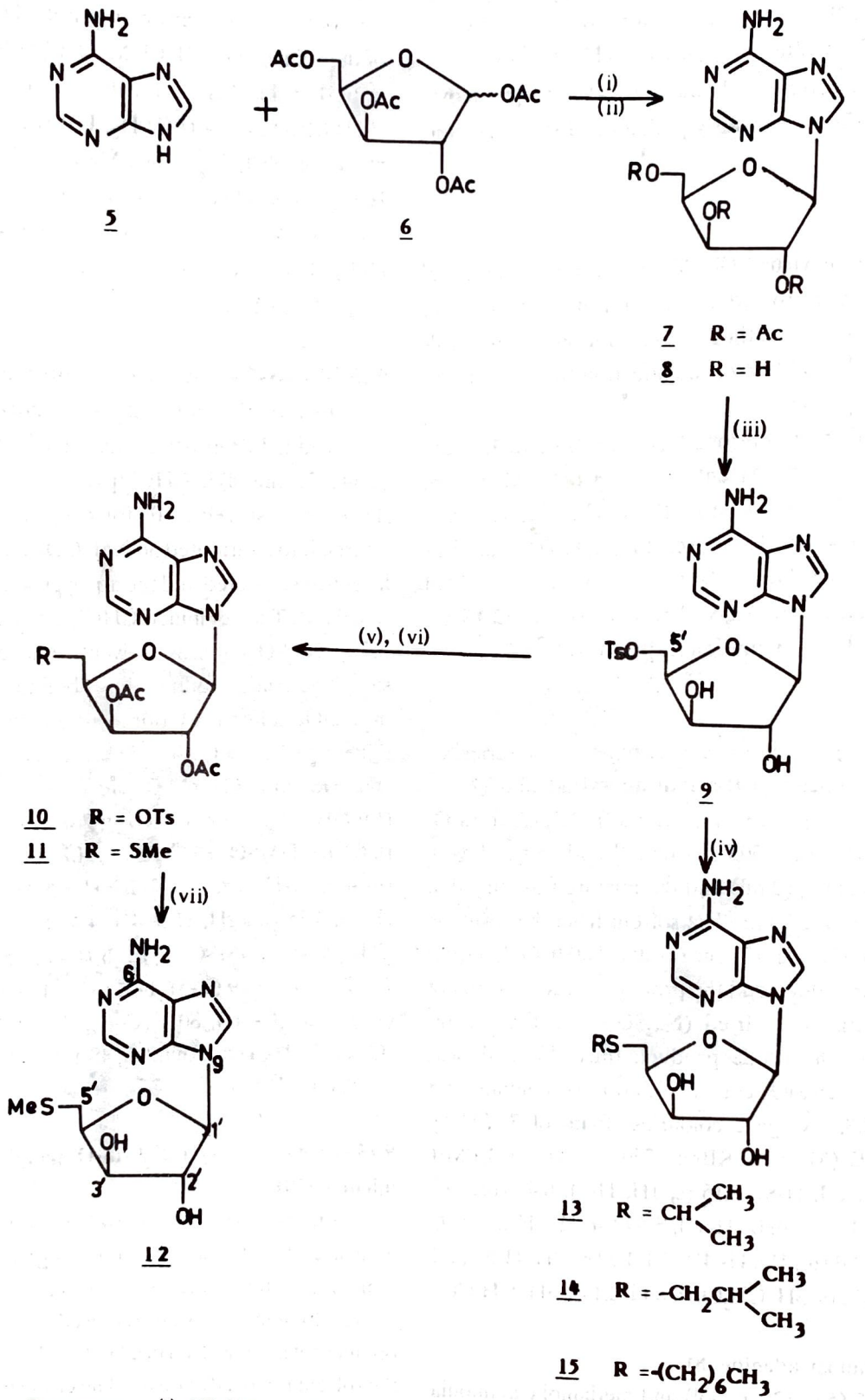
Fig. - 1



Reagents:- (i) CH_3NO_2, $SnCl_4$; (ii) MeOH-NH_3; (iii) TsCl-py, Δ; (iv) DMF, NaOMe, RSH, Δ; (v) Ac_2O-py; (vi) MeSNa, DMF, Δ; (vii) MeOH-NH_3.

Scheme - 1

Treatment of the key intermediate **9** with isopropyl, isobutyl and n-heptyl mercaptans separately afforded 9-[5′-deoxy-5′-(isopropylthio)-β-D-xylofuranosyl]adenine (**13**),9-[5′-deoxy-5′-(isobutylthio)-β-D-xylofuranosyl]adenine (**14**) and 9-[5′-deoxy-5′-(n-heptylthio)-β-D-xylofuranosyl] adenine (**15**) respectively in fairly good yields.

Antiviral assay

Ranikhet disease virus (RDV) was used for antiviral screening of the compounds. The strain of RDV, the haemaglutination test, the method of preparation of CAM culture and the optimal condition of the infection by the virus are described in earlier publication[19].

Aqueous solution/suspensions (0.1 mg/ml) of **12-15** were separately incubated in CAM culture using 6 CAM culture samples along with 0.064 HA/ml of RDV. The cultures were incubated at 37° for 48 hr. The percentage inhibition of virus multiplication was assayed from HA titre of the nutrient fluid of CAM culture infected with RDV. The compounds **12-15** exhibited 16, 0, 20, and 18% inhibition respectively.

Experimental

For experimental details see earlier paper in the series[20].

9-(2′,3′,5′-Tri-O-acetyl-β-D-xylofuranosyl)adenine (7)

To a mixture of **5** (135 mg, 1mmol), 1,2,3,5,-tetra-O-acetyl-D- xylofuranose (**6**, 0.505 g, 1 mmol) and anhy. $MeNO_2$ (50 ml) was added $SnCl_4$ (2 ml) and the mixture was stirred at ambient temperature for 20 hr. The solvent from the resulting mixture was removed *in vacuo*.To the residue, $NaHCO_3$ (600 mg) and H_2O (4 ml) were added and the product extracted with hot $CHCl_3$, washed with H_2O, dried (Na_2SO_4) and the solvent removed *in vacuo*. The crude product, thus obtained, was chromatographed over SiO_2 column. Elution of column with $CHCl_3$:MeOH (97:3, v/v) gave colourless foam of **7** (0.38g, 70%); MS: m/z 393 (M^+); IR(KBr): 1750 (C=O);PMR ($CDCl_3$): δ 8.35 (s, 1H, H-8), 8.15 (s, 1H, H-2), 6.45 (bs, 2H, NH_2), 6.25 (d, 1H, $J_{1'2'}$=3.0Hz, H-1′), 5.59 (m, 1H, H-2′), 5.45 (m, 1H, H-3′), 4.5-4.4 (m, 1H, H- 4′), 4.4-4.2 (m, 2H, H-5′), 2.2 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), and 2.1 (s, 3H, CH_3CO).

9-β-D-xylofuranosyladenine (8).

A mixture of **7** (5 g, 12.7 mmol) and methanolic ammonia (100 ml) (MeOH at 0° saturated with NH_3) was kept at ambient temperature for 24 hr. The solvent and excess of reagent from the resulting mixture were removed *in vacuo*. The crude product, thus obtained, was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (90:10, v/v) gave **8** (2.5 g, 74%); m.p. 135-40° (MeOH) (lit[21]., m.p. 125-40°); MS: m/z 267 (M^+);IR(KBr) : 3400 (O-H); UV (MeOH) : 258.4nm; (NaOH) : 258.4 nm; (HCl): 258.0 nm; PMR ($CDCl_3$ + DMSO-d_6);δ8.34 (s,1H, H-8); 8.28 (s,1H,H-2), 7.15 (bs, 2H, NH_2), 5.99 (d,1H,$J_{1'2'}$ = 3Hz,H-1′), 4.5 (m,1H,H-2′), 4.28 (m, 2H, H-3′,4′), 3.85 (d, 2H, J = 4.8 Hz, H-5′). (Found : C, 44.6; H, 4.5; N, 26.5 $C_{10}H_{13}N_5O_4$ requires C, 44.9; H, 4.9; N, 26.2%).

9-[5-′-Deoxy-5′-(tosyl)-β-D-xylofuranosyl]adenine (9)

To a solution of **8** (1.2g, 4.8 mmol) in pyridine (94 ml) at 0-5° was added dropwise a solution of *p*-toluenesulfonyl chloride (1.44 g, 7.5 mmol) in $CHCl_3$:pyridine (1:1, 36 ml) during 1 hr and the mixture stirred at 0° for 8 hr. The resulting mixture was poured into a saturated aq $NaHCO_3$ solution at 0-4°. The $CHCl_3$ layer was separated and the aq layer was extracted with $CHCl_3$(2 × 20 ml). The combined $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4) and the solvent removed under reduced pressure. The crude product, thus obtained, was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (92:8, v/v) gave **9** (1.04 g, 51%); crystallized from $CHCl_3$, m.p. 160°;MS: m/z 421 (M^+); IR (KBr): 3200, 1695, 1610; UV (MeOH): 259.2 nm; (NaOH): 260 nm; (HCl): 258.4 nm; PMR ($CDCl_3$ + DMSO-d_6): δ 8.3(s ,1H,H-8), 8.1 (s, 1H, H-2), 7.2-7.8 (m, 4H, Ar-H) , 7.1 (bs, 2H, NH_2), 5.9 (d, 1H, H-1′), 4.2 (m, 2H, H-5′), 4.35 (m, 2H, H-3′,4′), 4.45 (m, 1H, H-2′), 2.35 (s, 3H, CH_3); CMR (DMSO- d_6):156 (C-6), 152.4 (C-2), 148.7 (C-4), 139.7 (C-8), 118.9 (C-5), 145, 132.1, 129.9, 128.1, 127.6 and 125.5 (Ar), 89.6 (C- 1′), 80.0 (C-4′), 79.6 (C-3′), 75.3 (C-2′), 69.9 (C-5′), 21.0 (CH_3);(Found:C, 48.1; H, 4.8 $C_{17}H_{19}N_5O_6S$ requires C, 48.4; H, 4.5%).

9-[5′-Deoxy-5′-(tosyl)-2′,3′-di-O-acetyl-β-D-xylofuranosyl] adenine (10)

A mixture of **9** (0.2 g, 0.47 mmol),pyridine (10 ml), acetic anhydride (3 ml) and DMAP (0.05 g) were stirred at ambient temperature for 20 hr. Excess of reagent and the solvent from the resulting mixture were removed *in vacuo*. The residue thus obtained, was disssolved in $CHCl_3$ and dried (Na_2SO_4) and the solvent removed *in vacuo*. The crude product, thus obtained, was chromatographed over SiO_2. Elution of the column with $CHCl_3$:MeOH (98 : 2, v/v) afforded **10**, as a colourless foam (0.15

g, 61%); IR (neat): 1750 (CH_3CO); PMR ($CDCl_3$):δ8.8 (s, 1H, H-8), 8.3 (s, 1H, H-2), 7.55-7.8 (m, 2H, Ar-H),7.1-7.38 (m, 2H, Ar-H), 6.3 (d, 1H, $J_{1',2'}$ =3Hz, H-1'), 5.4-5.7 (m, 2H, H- 3',2'), 2.38 (s, 3H, $COCH_3$), 2.34 (s, 3H, CH_3), 2.15 (s, 3H, $COCH_3$).

9-[5'-Deoxy-5'-(methylthio)-2',3'-di-O-acetyl-β-D-xylofuranosyl] adenine (11)

A mixture of **10** (0.4g, 0.79 mmol), DMF (14 ml) and CH_3SNa (1g) were stirred at 80° for 24 hr. The resulting mixture was cooled and neutralized with dil acetic acid and the solvent removed *in vacuo*. The crude product, thus obtained, was passed through sephadex column. Elution of the column with MeOH afforded **11** as an oil (0.15g, 50%); IR (Neat): 1750 (C=O); PMR ($CDCl_3$): δ8.5 (s, 1H, H- 8), 8.2 (s, 1H, H-2), 6.2 (d, 1H, $J_{1',2'}$ =3Hz, H- 1'), 5.4-5.7 (m, 2H, H-2',3'), 4.25-4.45 (m, 2H, H-5'), 4.5-4.7 (m, 1H, H-4'), 2.5 (s, 3H, SCH_3), 2.5 (s, 3H, SCH_3), 2.11 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$).

9-[5'-Deoxy-5'-(methylthio)-β-D-xylofuranosyl]adenine (12)

A mixture of **11** (0.2 g, 0.52 mmol) and methanolic ammonia (MeOH at 0° saturated with NH_3) was kept at ambient temperature for 25 hr. The solvent and excess of reagents from the resulting mixture were removed *in vacuo*. The crude product, thus obtained, was passed through sephadex column. Elution of the column with MeOH afforded **12** (90 mg, 60%); base picrate m.p. 197-98° (Lit[17]. picrate m.p. 199-201°); MS: m/z 297 (M^+); IR (KBr): 3400; UV (MeOH): 258 nm; (NaOH): 257 nm; (HCl): 258 nm; PMR (DMSO-d_6): δ8.2 (s, 1H, H-8), 8.1 (s, 1H, H-2), 7.32 (bs, 2H, NH_2), 6.15 (d, 1H, J = 8Hz, OH), 5.95 (d, 1H, J =6.4 Hz, OH), 5.82 (d, 1H, $J_{1',2'}$ =1.6 Hz, H-1'), 4.2-4.3 (m, 2H, H- 2',4'), 3.96 (m, 1H, H-3'), 2.6-2.85 (dd, 2H, J=8.12, H-5'), 2.15 (s,3H, SCH_3); (Found : C, 44.5; H, 4.9; N, 23.4 $C_{11}H_{15}N_5O_3S$ requires C, 44.4; H, 5.05; N, 23.5%).

9-[5'-Deoxy-5'-(isopropylthio)-β-D-xylofuranosyl]adenine (13)

A mixture of **9** (0.5g, 1.2 mmol), DMF (20 ml), NaOMe (0.5g) and isopropylmercaptan (1 ml) were heated at 80° for 8 hr, cooled and then neutralized with dil acetic acid. The solvent and excess of reagents were removed *in vacuo*. The curde product, thus obtained was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (95:5, v/v) afforded **13** (0.2 g, 52%); m.p. 90°, crystallization from MeOH; MS: m/z 325 (M^+);IR(KBr): 3400;PMR(DMSO- d_6):δ 8.2 (s, 1H, H-8), 8.1(s, 1H, H-2), 7.3 (bs, 2H, NH_2), 5.9 (d, 1H, H-1'), 4.5-4.3 (m, 1H,H-2'), 4.2-4.0(m, 1H, H-4'), 3.6-3.2 (m, 1H, H-3'), 3.0-2.8 (m, 2H, H-5'), 2.1 (m, 1H, -CH), 1.25 (d, 6H, 2 CH_3,J =6Hz); (Found : C, 48.3; H, 6.1; N, 21.3 $C_{13}H_{19}N_5O_3S$ requires C, 48.0; H, 5.8; N, 21.5%).

9-[5'-Deoxy-5'-(isobutylthio)-β-D-xylofuranosyl]adenine (14).

A mixture of **9** (0.5g, 1.2 mmol) DMF (25 ml), NaOMe (0.5 g) and 2-methyl-1-propanethiol (5 ml) were heated at 80° for 8 hr. It was then cooled to ambient temperature and neutralized with dil acetic acid. The excess of solvent and reagents were removed *in vacuo*. The crude product, thus obtained, was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (95:5, v/v) afforded **14** (0.18 g, 45%) as semi solid; MS: m/z 339 (M^+); IR (KBr): 3400; PMR (DMSO-d_6):δ8.3 (s, 1H, H-8), 8.25 (s, 1H, H-2), 7.4 (bs, 2H, NH_2), 6.0 (d, 1H, H-1'), 4.2-4.6 (m, 3H, H- 2',3',4'), 2.8-3.0 (m, 2H, H-5'), 2.6(d, 2H, CH_2), 2.1 (m, 1H, CH-), 1.05 (d, 6H, 2 CH_3, J =6Hz); (Found : C, 49.8; H, 6.5; N, 20.8 $C_{14}H_{21}N_5O_3S$ requires C, 49.5; H, 6.2; N, 20.6%).

9-[5'-Deoxy-5'-(n-heptylthio)-β-D-xylofuranosyl]adenine (15)

To a mixture of **9** (0.5 g, 1.2 mmol),DMF (25 ml), NaOMe (0.5 g) was added n-heptyl mercaptan (5 ml). The mixture was stirred at 80° for 8 hr, cooled and neutralized with dil acetic acid. The solvent and excess of reagents were removed *in vacuo*. The crude product, thus obtained, was chromatographed over SiO_2 column. Elution of the column with $CHCl_3$:MeOH (94:6, v/v) gave **15** (0.26g, 57%); crystallization from MeOH, m.p. 145°; MS: m/z 381 (M^+); IR(KBr): 3400; PMR ($CDCl_3$ + DMSO-d_6):δ8.1 (s, 1H, H-8), 8.0 (s, 1H, H-2), 7.1 (bs, 2H, NH_2), 5.7 (d,1H,H-1'), 4.4-4.1 (m, 3H,H-2',3',4'), 2.5 (m, 2H, H-5'), 1.1- 1.3 (m, 15H, C_7H_{15});(Found : C, 53.8; H, 7.3; N, 18.1 $C_{17}H_{27}N_5O_3S$ requires C, 53.5; H, 7.08; N,18.3%).

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