Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1789–1800

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Antifilarial Evaluation of N^1,N^n- Xylofuranosylated Diaminoalkanes†

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Received 22 August 2002; accepted 13 December 2002

Abstract—A series of N^1,N^n-xylofuranosylated diaminoalkanes (**3**–**9** and **11**–**18**) has been synthesized either by reductive amination of deoxy xylouloses (**2a**, **2b**) with amines followed by one pot reduction with $NaBH_4$ or $NaCNBH_3$; or by 1,4-conjugate addition of amines to glycosyl olefinic esters (**10a**, **10b**). The compounds were screened for their interference with filarial worms' glutathione metabolism, a potential target for chemotherapeutic attack. Interestingly, these compounds affected intracellular glutathione, γ-glutamyl cysteine synthetase, glutathione reductase and glutathione-*S*-transferase(s) of bovine filarial worms to varying degrees. Some of the compounds though effected the motility and MTT reduction potential of filarial worms *Brugia malayi*, however, little microfilaricidal and macrofilaricidal were noted with compounds at 50 mg/kg oral dose. Compounds **6**, **16** and **17** were evaluated also for in vivo activity.

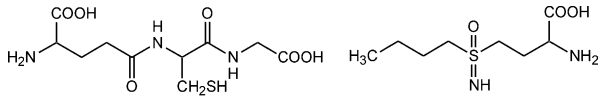
Introduction

The disease filariasis, caused by filarial worms, is a major public health and socioeconomic problem in most of the developing countries. The identified filarial worms infecting humans are *Brugia malayi, Onchocerca volvulus, Loa loa, Acanthocheilonema perstans, Acanthcelonema streptocerca, Mansonella ozzardi*. To date, more than 700 million people are infected with lymphatic filariasis and about 150 million people in world are living in endemic areas of filariasis.[1–3] An estimated population of 22 million is known to be host for circulating microfilarie and the 16 million people suffer from filarial manifestations like elephantiasis of limbs, genitals and hydrocoele. About 120 million people are infected with one or the other forms of the filariasis.[1–3]

The effective treatment regimens to decrease microfilaremia have been primarily responsible for the recent designation by the World Health Assembly of lymphatic filariasis as a disease that can be eliminated globally.[3]

Though diethyl carbamazine (DEC) and ivermectin are available for filarial chemotherapy, both are mainly microfilaricidal and are associated with one or more drawbacks.[3] Presently identification of novel therapeutic targets from the myriad of parasite enzymes, receptors, genome data and metabolic pathways[4] and development of new active chemical entities against these enzymes are the key goals in drug discovery process.[5] The antiparasitic agents known till date either act on the membrane receptors or on enzymes/metabolic pathways crucial for the survival of the parasite (Fig. 1).[6]

Glutathione (**1a**, GSH), a tripeptide consisting of glutamic acid, cysteine and glycine is known to guard the filarial worms from oxidative stress and attack by reactive oxygen species (ROS) as well as electrophilic toxic compounds. Its role in modulation of drug resistance has also been currently established.[6a] The possible role



1a, GSH **1b**, BSO

Figure 1.

†CDRI communication No. 6298.
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doi:10.1016/S0968-0896(03)00033-6

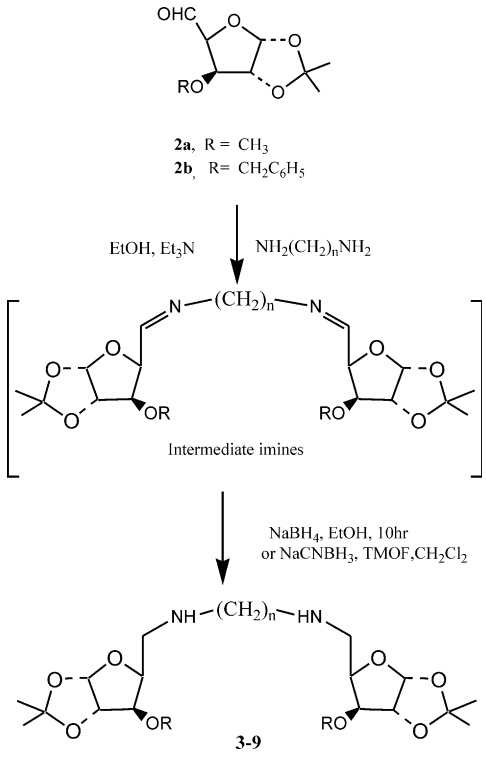
of GSTs (glutathione-*S*-transferase) in protection of filarial worms from oxidative stress and eicosanoid production (the immunopathogenic components) also makes it a vulnerable chemotherapeutic target in developing new drugs. Intracellular GSH level depends on numerous factors but predominantly on two step de novo synthesis from the above three amino acids, and on efficient reduction of glutathione disulphide (GSSG) by glutathione reductase (GR). Glutathione is synthesized both in eucaryotes and prokaryotes by sequential action of γ-glutamylcysteine synthetase (γ-GCase) and GSH synthetase.[6b,7] Pharmacological depletion of GSH in the cells is known to be useful in the development of anticancer drugs and the increased vulnerability of the infected cells due to internally generated oxidants is useful in the development of antiparasitic agents[8,9] The potent inhibitors of GR enzyme, BSO (**1b**, L-buthionine sulfoximine) and certain dyes have been used to block GSH synthesis and to promote GSH depletion. Many inhibitors of enzymes involved in GSH metabolism are known to be associated with antiparasitic activities however they are associated with many drawbacks too. Both available filaricides DEC and ivermectin are able to eliminate microfilaraemia in human lymphatic filariasis,[7] however, the adult parasites mostly remain unaffected with the result there is relapse of microfilaraemia and no relief from the filarial disease lesions. Therefore, an antifilarial drug is required which can kill the adult parasites slowly, has microfilaricidal activity and at the same times sterilize the female worms.

In an our ongoing study towards the development of novel mechanism based antifilarial agents, it is shown that certain sugar derivatives posses very good activity against filarial glutathione metabolizing enzymes in vitro.[10–12] These compounds possess immunomodulatory activity[13] and in vitro antifilarial activity too. As sugars are known to offer better pharmacokinetics, better transport and above all less toxicity[14,15] to the drugs, it is presumed that amino acid analogues if grafted on sugar backbone may serve as substrate for GSH biosynthesis and may hamper its de novo synthesis while at the same time invoking the immunological response of the host to boost up the defence system of the host in parasitic infections. Keeping in view the above facts we have synthesized the title compounds and evaluated their effect on intracellular GSH content of bovine filarial worms *Setaria cervi* during in vitro maintenance and on isolated γ-GCase, GR and GSTs from *S. cervi*. Their in vitro antifilarial efficacy were determined against human filarial worm *B. malayi* and three compounds were also looked for their antifilarial efficacy in vivo.

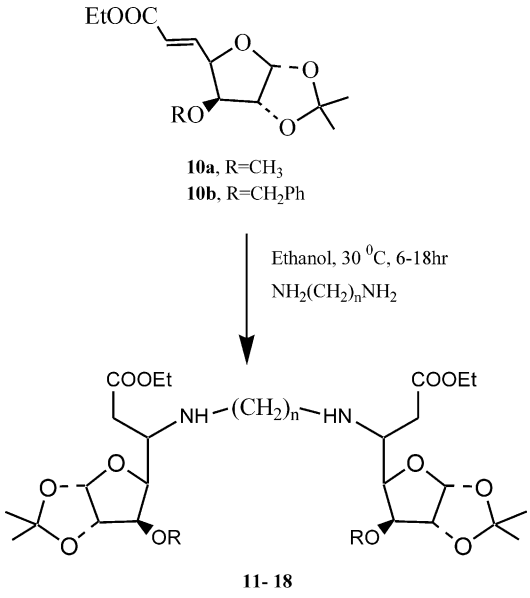
Results and Discussion

Chemistry

The starting material 3-*O*-benzyl/methyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-uloses (**2a** and **2b**) and 3-*O*-benzyl/methyl-1,2-*O*-isopropylidene-α-D-xylofuranosyl olefinic esters (**10a** and **10b**) were obtained from D-glucose according to the procedure reported in literature.[16,17] The synthesis of the desired N^1,N^n-diglycosylated diaminoalkanes is presented in Schemes 1 and 2. N^1,N^n-bis-[5-deoxy-1,2-*O*-isopropylidene-3-*O*-benzyl/methyl-α-D-xylofuranos-5-yl]-N^1,N^n-diaminoalkanes (**3–9**) were obtained by reductive amination of aldehydes **2a** and **2b** with different amines including 1,3-diamino propane, 1,7-diamino heptane, 1,10-diamino decane and 1,12-diamino dodecane using $NaBH_4$ or $NaCNBH_3$. Thus, two moles of 3-*O*-benzyl (methyl)-1,2-*O*-isopropylidene-5-uloses (**2a** and 2**b**) on reaction with one mole of the above diaminoalkanes separately in presence of triethylamine



Scheme 1.



Scheme 2.

led to the corresponding imines which on further one pot reaction either with sodium borohydride or sodium cyanoborohydride gave corresponding N^1,N^n-diglycosylated diaminoalkanes (**3–9**) in quantitative yield. That the imines are intermediates in the above reaction was proved with the isolation and characterisation of one such imine in the reaction of **2b** with 1,7 diamino heptane. Conversion of aldehyde into imine in the reaction could be monitored by ^{1}H NMR spectrum where the disappearance of a singlet at δ 9.5 (CHO) and appearance of a doublet at δ 7.70 (imine proton) having J value 4.1 Hz were noticed.

The glycosylated amino esters **11–18** were obtained by the conjugate addition of one mole of the above diamines to the two moles of glycosyl olefinic esters, (*E*) ethyl [3-*O*-methyl (benzyl)-5,6-dideoxy-1,2-*O*-isopropylidene]-α-D-1, 4-heptofuranosyl-5-enoate (**10a** and **10b**). The latter were prepared from the above xylouloses **2a** and **2b** by the method reported by us.[17] Thus, 1,4-conjugate addition of diamines to the esters **10a** and **10b**, separately led to the corresponding bisxylofuranosylated diaminoalkanes **11–18** in good yield.

The above conjugate addition of diamines to the olefinic ester was diastereoselective and in each reaction a diastereomeric mixture of three compounds, that is compound having β-L-*ido* (*S*) configuration at C-5 in both sugar units, compound having β-L-*ido* (*S*) configuration at C-5 in one sugar and α-D-*gluco* (*R*) configuration in the other sugar portion and compound having α-D-*gluco* (*R*) configuration at C-5 in both sugar units. The formation of diastereoisomers was evident from TLC and ^{1}H NMR spectral data. The former two isomers were isolated in few reactions by column chromatography, however, the latter diastereomer could not be isolated pure.

Ratios of isomers were determined by ^{1}H NMR spectra and by HPLC method using a chiral column and are depicted in Table 1.

The configuration at C-5 in diastereoisomers was determined on the basis of ^{1}H NMR spectra. It is known that in C-5 epimeric pair, derived from D-glucofuranose, $J_{4,5,}$ observed in diastereomeric mixture is always higher in the L-*ido*-(C-5 with '*S*' configuration) isomer than in the D-*gluco*-(C-5 with '*R*' configuration) isomer.[17] Further, comparison of the chemical shift of H-3 in both isomers is also reported to be diagnostic in deciding the stereochemistry at C-5 in such epimeric pair where it is significantly upfield in the L-*ido* isomer as compared to D-glucoisomer. Taking into account the ^{1}H NMR spectra of epimeric pair **15a** and **15b**, the chemical shifts for H-3 in **15a** in one sugar ring is at δ 4.20, while at δ 3.9 in the other sugar ring; however in **15b** H-3 appears at δ 3.9 only. Further, in **15a** $J_{4,5}$ is 6.7 Hz while in **15b** it is 8.8 Hz. Thus in **15a** one sugar has D-*gluco*-(C-5 '*R*') configuration and the other one has L-*ido* (C-5 '*S*') configuration, as accordance with the literature.

Table 1. N^1,N^n-Bis-xylofuranosylated diaminoalkanes

Compd	R	*n*	Ratios of isomers (RR, RS, SS)
3	CH_3	3	na
4	CH_3	7	na
5	CH_3	10	na
6	CH_3	12	na
7	$CH_2C_6H_5$	7	na
8	$CH_2C_6H_5$	10	na
9	$CH_2C_6H_5$	12	na
11	CH_3	3	6:45:49
12	CH_3	7	5:45:50
13	CH_3	10	5:45:50
14	CH_3	12	4:46:50
15	$CH_2C_6H_5$	3	5:35:60
16	$CH_2C_6H_5$	7	4:36:60
17	$CH_2C_6H_5$	10	4:35:61
18	$CH_2C_6H_5$	12	4:35:61

na, not applicable.

The data about biological activity are given for diastereomeric mixture only. There was no significant improvement in efficacy when pure diastereoisomers were tested individually.

Antifilarial activity

Table 2a demonstrates the effect of various concentrations of BSO (10–250 μM) on intracellular GSH of *S. cervi* females. BSO deplete intracellular *S. cervi* GSH in dose dependent manner. 10, 50 and 250 μM BSO concentration deplete around 11, 26 and 36% of GSH respectively after treatment of the worms for 6 h at 37 °C.

Table 2b represents the effect of Carmustine (RCNU) on GSH content in S. cervi females at various concentrations. 10, 50 and 250 μM Carmustine depletes around 19, 35 and 69% of intracellular *S. cervi* GSH, respectively.

Table 2a. Effect of BSO on GSH content in *S. cervi*[a]

BSO (μM)	mg GSH/g protein[b]	% GSH depletion
0.0	5.927±2.037	Control
10	5.289±1.580	10.76
50	4.382±2.215	26.06
250	3.817±1.864	35.60

[a]The parasites were incubated in vitro with varying concentrations of BSO for 6 h at 37 °C.
[b]Mean values±SD for three independent experiments done in duplicate.

Table 2b. Effect of carmustine on GSH content in *S. cervi*[a]

BCNU (μM)	mg GSH/g protein[b]	% GSH depletion
0.0	4.599±0.663	Control
10	3.740±0.406	18.67
50	2.979±1.259	35.22
250	1.427±0.100	68.97

[a]The parasites were incubated in vitro with varying concentrations of BCNU for 6 h at 37 °C.
[b]Mean values±SD for three independent experiments done in duplicate.

Table 2c illustrates the effect of increasing concentration of Menadione on GSH depletion. 10 μM Menadione depleted 44.4% GSH whereas 50 and 250 μM depleted GSH around 77.2 and 96%, respectively.

Table 2d depicts the results obtained with compounds **3**–**9** and **11**–**18**. Compounds **3**, **4**, **6**, **7**, **14** and **18** increase the intracellular GSH content to the tune of 18.1, 52.5, 38.8 69.0, 37.0 and 66.7%, respectively. However, examples **8**, **9**, **15** and **17** showed the reverse effect. The respective percent decline in these cases were 52.5, 59.7, 57.8 and 58.2%. Except compounds **14** and **18**, compounds **11**–**18** declined the intracellular GSH content. Compounds **11** and **16** showed mild effect (14–19%), whereas compounds **5**, **12** and **13** showed moderate effect. Table 2d also depicts the effects of compounds **3**–**9** and **11**–**18** on γ-GCase, GR and GST. Nearly all the compounds inhibit γ-GCase activity. The maximum effect was noted with compound **5**, followed by compound **16**, whereas all other compounds showed around 10–30 inhibitory effect. Compounds **3**, **4**, **5**, **6**, **8**, **11**, **13**, **15**, and **18** inhibited GR activity to the tune of 18–30%. Except compound **14**, all other compounds either stimulated GST or did not show any remarkable effect. The percent stimulation was maximum with compound **16**, followed by **18**. BSO and Cystamine inhibited γ-GCase activity to around 17 and 66%, respectively, whereas menadione did not show any significant effect on γ-GCase. Menadione and carmustine showed stimulatory effect on GR activity. The percent stimulation were around 122 and 54, respectively. All the four inhibitors, that is BSO, Cystamine, carmustine and menadione showed stimulatory effect on GST. BSO stimulated GST around 76%, whereas nearly 20–30% stimulation on GST was noted with the other three inhibitors.

Table 2c. Effect of menadione on GSH content in *S. cervi*[a]

Menadione (μM)	mg GSH/g protein[b]	% GSH depletion
0.0	4.833±0.622	Control
10	2.688±0.402	44.39
50	1.103±0.190	77.17
250	0.191±0.016	96.04

[a]The parasites were incubated in vitro with varying concentrations of Menadione for 6 h at 37 °C.
[b]Mean values±SD for three independent experiments done in duplicates.

Diamines as such did not exhibit any significant activity in vitro either against the enzymes or motility or MTT reduction assays. On careful analysis of the biological activity data it is seen that the biological activity is independent of C-carbethoxy-methyl substituent as unsubstituted glycosylated diamines are equipotent to those having C-carbethoxymethyl substituent. Further, activity is also independent of 3-*O*-substituent in sugar ring as compounds having 3-*O*-methyl substituent with a 12 carbon diamine (**6**) and 3-*O*-benzyl substituent compound with 7 carbon diamines (**16**) were most active. Among 5-carbethoxymethyl substituents, 3-*O*-benzyl derivatives with 7 carbon diamine fragment was found to be most active. Thus with 3-*O*-methyl substituent a 12 carbon diamine spacer and with 3-*O*-benzyl substituent 7 carbon diamine spacer are required to show maximum activity.

In effective sugar derivatives **6** and **16** lipophilic diamine spacers and 3-*O*-substituent along with 1,2-*O*-isopropylidene group probably help the compounds to bind with the active sites of enzymes or interfere with parasite metabolic machinery (Table 3).

Efficacy in *B. malayi* in *M. coucha* model

The recovery of adult worms from the animals treated with compound **14** at 50 mg/kg ip was lesser (38.7% adulticidal action) than control untreated animals with

Table 2d. Effect of title compounds **3**–**9** and **11**–**18** on γ-GCase, GR, GST and intracellular GSH pool

Compd	% Modulatory effect			
	γ-Gcase[a]	GST[b]	GR[a]	GSH[c]
3	18.4±0.99	4.6±0.14	+18.9±3.14	+18.2±2.1
4	21.1±0.141	+16.0±1.8	18.0±1.9	+52.5±5.8
5	56.9±27.7	12.0±1.1	26.0±3.4	−32.7±6.9
6	27.8±5.6	+49.0±2.8	24.0±3.2	+38.8±19.5
7	36.2±7.7	+57.9±3.3	Nil	+69.3±1.2
8	20.4±1.41	+45.8±1.3	+30.6±7.1	−52.5±9.6
9	31.0±0.721	+71.0±9.9	Nil	−59.7±13.7
10	12.0±0.16	+2.4±0.6	26.0±1.8	−15.8±11.3
11	31.2±3.6	6.9±0.07	Nil	−39.0±28.8
12	28.1±3.65	+36.8±8.2	+18.4±0.78	−47.9±11.5
13	24.4±6.5	44.0±2.3	Nil	+37.1±8.7
14	11.57±0.03	+19.0±1.3	24.0±1.2	−57.8±8.9
15	53.46±0.94	+140±2.1	Nil	−15.9±7.5
16	30.1±8.2	+43.5±0.85	Nil	−58.2±22.4
17	15.8±7.7	+102.7±3.9	+21.1±12.6	+66.7±46.4
BSO	17.4±2.6	+75.6±7.9	—	—
Cystamine	66.4±0.39	+20.6±0.99	—	—
Menadione	79.5±4.7	+29.4±6.6	+121.7±21.1	—
Carmustine	70.4±2.2	+25.6±1.3	+54.05±17.9	—

[a]Concentration of test sample/standard inhibitor in the assay system 200 μM.
[b]Concentration of test sample/standard inhibitor in the assay system 100 μM.
[c]Concentration of test sample/standard inhibitor in the assay system 250 μM.

Table 3. In vitro effect of compounds **3–9** and **11–18** on lymphatic filarial worms *Brugia malayi*

Compd	Effect on lymphatic filarial worms In vitro	
	Motility[a]	MTT
3	+2	Nil
4	+3	Nil
5	+2	Nil
6	0	62.0±3.7
7	+3	Nil
8	0	50.6±9.5
9	0	94.2±0.8
11	0	35.3±13.8
12	+3	Nil
13	+2	39.5±0.1
14	+3	Nil
15	+3	Nil
16	0	Nil
17	+3	52.9±0.1
18	+3	Nil
Ivermectin	Nil	Nil
DEC	Nil	Nil

Nil, no inhibition.
[a]Motility score: +3 (active); +2 (sluggish); +1 (paralysed); 0 (dead).

63.80% sterilization action on female parasites. The reduction in microfilaraemia registered was 42.4% (microfilaricidal activity) on the first week [day 8 post treatment (pt)] of treatment. The maximum microfilaricidal efficacy was found on the ninth week pt. However, on the 13th week pt the mf count increased and almost touched the pretreatment level showing 13.6% microfilaricidal action only. Compounds **16** and **17** were ineffective against mf or adult parasites (Table 4).

Ivermectin at 2 mg /kg (sc) caused 50.1% reduction in microfilaraemia on the first week and thereafter the mf count remained suppressed between (39.7 and 67.9%) till the day of sacrifice. More than 50% adult worms were cleared and 68.3% of the female worms recovered were sterile (Table 4). Untreated control animals showed progressive rise in mf count with 26.1% sterilization of female worms (Table 4).

Efficacy in *B. malayi* in jird (*M. unguiculatus*) model

Against *B. malayi* in jird (adult worm transplanted infection) the intraperitoneal microfilariae in the *M. unguiculatus* were unaffected with the compound **14** at 50 mg/kg, × 5 days. Lesser number of adult parasites were recovered from the animals treated with compound **14** (33.5% adulticidal action) with 50.0% sterilization effect on females worms (Table 5). Ivermectin (2 mg/kg, sc×5 days) did not kill the adult parasites in jirds. However, 77.1% of the females were sterile. Control untreated animals showed negligible sterilization of female worms (Table 5).

Conclusion

It is concluded from the above study that bisxylofuranosylated diamino alkanes showed varying effects on filarial enzymes in vitro. Further, this class of compound result in complete loss of motility and at the

Table 4. Antifilarial efficacy of compounds against *Brugia malayi* in rodents (values in mean±SD)

Compd. (no. of animals used)	Mf count/10 μL of blood (week post treatment)								Adult worm burden			% Sterilization of female worms
	0 (before)	1	3	5	7	9	11	13	Female	Male	Total	
Control, untreated (4)	60.75±18.46	65.75±21.39	92.75±24.31	117.5±45.49	115.25±38.62	116.75±36.59	115.75±45.04	108.5±39.33	14.75±3.59	4.5±1.29	19.25±2.38	23.44±16.64
14 (5)	72.60±59.09	38.20±28.18	58.0±26.56	51.0±27.27	26.0±18.12	45.0±31.41	36.80±31.13	68.0±38.89	9.0±1.87	2.8±1.09	11.8±1.64	63.80±22.76
Control, untreated (2)	102.0±104.6	91.0±100.41	89.0±57.9	126.5±2.1	105.0±11.3	142±20.5	131±7.1	132±8.5	19.0±7.07	9.5±0.71	28.5±7.78	39.3±15.2
16 (2)	58±2.8	42.5±13.4	45.5±3.5	51±7.1	58.5±19	36.5±2.1	47.5±19.1	17.1±41	17.0±2.83	7.0±0	24.3±1.5	45.32±8.93
17 (2)	43.5±12	32.5±10.6	52±28.3	41.5±26.1	101±86	57±35	63.5±40	ND	14.0±5.66	7.5±0.71	21.5±4.95	54.45±21.99
Control, untreated (3)	65.33±34.36	102.67±56.45	138.0±62.0	189.0±121.15	207.33±98.19	232.67±63.22	216.33±45.00	282.33±64.93	9.00±3.61	5.67±1.53	14.67±4.93	26.11±6.73
Ivermectin (2 mg/kg, sc×5 days) (3)	46.33±29.57	29.0±27.0	20.0±18.03	13.0±5.57	22.33±13.58	22.33±14.84	19.33±6.65	11.33±1.53	4.67±1.53	2.0±1	6.67±2.31	94.44±9.62

Table 5. Antifilarial efficacy of compound **14** against *Brugia malayi* in *Meriones unguiculatus*[a] (values in mean ± SD)

Antifilarial agent	No. of animals	Effect on microfilariae in peritoneal cavity	Adult worm recovery			
			Female	Male	Total	% Sterilization of female worms
Control (untreated)	2	No effect	7.5 ± 1.41	5.0 ± 0.71	12.5 ± 2.12	6.25 ± 8.84
Ivermectin[b]	2	No effect	8.4 ± 0.71	4.5 ± 0.71	13.0 ± 1.41	77.09 ± 14.73
Compound **14**[c]	3	No effect	5.33 ± 0.58	3.0 ± 1.0	8.33 ± 1.53	50.0 ± 30.00

[a]Transplanted with 10 female and five male adult parasites of *B. malayi*.
[b]Dose: 2 mg/kg, sc × 5 days.
[c]Dose: 50 mg/kg, ip × 5 days.

same time exhibit mild microfilaricidal, macrofilaricidal and sterilization effect. Thus, bisxylofuranosylated diaminoalkanes would emerge as lead molecule for further exploration to develop new class of antifilarial drug.

Experimental

Chemistry

All glasswares were dried in an open flame before use in connection with an inert atmosphere. Solvents were evaporated under reduced pressure and evaporation was carried out at temperature $<50\,^{\circ}C$. Thin layer chromatography was performed using silica gel 60 F_{254} plates with detecting agents iodine vapours, spraying with 5% sulphuric acid in ethanol followed by heating at 100 °C, or by spraying with Dragendorf reagent. Silica gel (60–120 mesh) was used for column chromatography. Tetramethylsilane (0.0 ppm) was used as an internal standard in ^1H NMR and $CDCl_3$ (77.0 ppm) was used in ^{13}C NMR. The abbreviations used to indicate the peak multiplicity were; s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; Hz, Hertz. FAB MS was recorded on Jeol (Japan)/SX-102. Infrared spectrum was taken with KBr on Perkin-Elemer RX-1. Melting points were determined on a Buchi 535 digital melting point apparatus and were uncorrected. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within ±0.4% of the calculated values. The optical rotations were measured in a 1.0 dm tube with Jasco dip-140 polarimeter in chloroform, methanol or ethyl acetate. Anhydrous sodium sulphate (Na_2SO_4) was used as drying agent for the organic phases containing the compounds. Unless otherwise stated, all materials were obtained from commercial suppliers Sigma Aldrich Company, Lancaster, SRL and Spectrochem Pvt. Ltd. and were used without further purification.

General procedure

***N*¹,*N*³-Bis-[5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,3-diaminopropane (3). Method A.** To a magnetically stirring solution of 3-*O*-methyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-ulose (**2a**, 2.0 g, 9.9 mmol) and 1,3-diaminopropane (0.42 mL, 5.00 mmol), in absolute ethanol (25 mL), Et_3N (2.5 mL) was slowly added at 0 °C and stirring continued for 0.5 h. The stirring reaction mixture was brought to ambient temperature and stirring continued till the disappearance of aldehyde. $NaBH_4$ (0.8 mmol) dissolved in absolute ethanol (10 mL) was added to stirring reaction mixture at 0 °C and stirring continued for 12 h. The reaction mixture was filtered and resulting solid cake was washed with more ethanol. The solvent was evaporated under reduced pressure and the residue, thus obtained, was quenched with a saturated aqueous NH_4Cl. The latter was extracted with ethyl acetate (2 × 100 mL) and washed with water (25 mL). The organic layer dried (Na_2SO_4) and evaporated under reduced pressure to give a crude mass which was chromatographed over SiO_2 column using chloroform/methanol (95:5) as eluant to give **3** as colorless oil. Yield 65%, $[\alpha]_D = -43.2$ (*c*, 0.06, $CHCl_3$), IR (KBr): ν cm^{-1} 3138 (–NH), 2980–2800 (CH_3 and CH_2 stretching); FABMS: 447 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 5.88 (d, $J = 3.8$ Hz, 1H, H-1), 4.56 (d, $J = 3.8$ Hz, 1H, H-2), 4.28 (m, 1H, H-4), 3.67 (d, $J = 3.1$ Hz, 1H, H-3), 3.41 (s, 3H, OCH_3), 2.91 (m, 2H, H-5), 2.62 (t, $J = 6.9$ Hz, 4H, 2×–NCH_2), 1.85 (s, 1H, –NH), 1.48 and 1.32 [each s, each 3H, 2× >C($C\underline{H}_3$)$_2$], 1.31(m, 2H, CH_2); ^{13}CNMR ($CDCl_3$): δ 112.0 [>$\underline{C}(CH_3)_2$], 105.18 (C-1), 84.6 (C-2), 82.10 (C-3), 79.84 (C-4), 58.0 (OCH_3), 50.32 (C-5), 47.18 (–$NH\underline{C}H_2$), 29.54 (–$NCH_2\underline{C}H_2$), 27.17 and 26.65 [2 × >C ($\underline{C}H_3$)$_2$]. Anal. calcd for $C_{21}H_{38}N_2O_8$: C, 56.50; H, 8.52; N, 6.28; Found: C, 54.13; H, 8.21; N, 5.95.

Method B. The above compound **2a** (1.0 g, 4.95 mmol) was dissolved in a mixture of trimethyl orthoformate (5 mL) and dichloromethane (10 mL) and stirred magnetically at 0 °C. 1,3-diaminopropane (0.21 mL, 2.5 mmol), was added and stirring continued for another 4 h at the same temperature. $NaCNBH_3$ (0.307 g, 4.45 mmol) was added to the stirring reaction mixture and stirring continued for 12 h at 30 °C. Solvent evaporated under reduced pressure and the residue was dissolved in ethyl acetate and washed with aquous NH_4Cl (2 × 20 mL) followed by water (2 × 15 mL). It was dried (Na_2SO_4) and evaporated under reduced pressure to give the crude mass, which was chromatographed over SiO_2 column as described above to give **3** as colourless oil (Yield 60%). The compound was found to be identical in all respects to that obtained in method A.

***N*¹,*N*⁷-Bis-[5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,7-diaminoheptane (4).** Reaction of 3-*O*-methyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-ulose (**2a**, 1.0 g, 4.9 mmol), 1,7- diaminoheptane (0.32 g, 2.48 mmol), Et_3N (1.5 mL) and $NaBH_4$ (0.10 g,

2.63 mmol) in absolute ethanol as above gave **4** as colourless oil. Yield 65%, $[\alpha]_D = -50.28$ (*c* 0.08, $CHCl_3$), FAB MS: *m/z* 503 $(M+H)^+$; IR (KBr): ν cm^{-1} 3134 (–NH), 2980–2800 (CH_3 and CH_2 stretching); ^1H NMR (200 MHz, $CDCl_3$): δ 5.89 (d, *J* = 3.8 Hz, 1H, H-1), 4.57 (d, *J* = 3.8 Hz, 1H, H-2), 4.29 (m, 1H, H-4), 3.69 (d, *J* = 3.1 Hz, 1H, H-3), 3.41 (OCH_3), 2.91 (m, 2H, H-5), 2.63 (t, *J* = 6.9 Hz, 4H, 2 × –NCH_2), 1.90 (bs, 1H, –NH), 1.49 and 1.32 [each s, each 3H, 2 × >C(CH_3)$_2$], 1.30 (m, 10H, CH'_2s); ^{13}C NMR ($CDCl_3$): δ 112.06 [>C(CH_3)$_2$], 105.21 (C-1), 85.02 (C-2), 82.14 (C-3), 79.90 (C-4), 58.12 (OCH_3), 50.48 (C-5), 48.28 (–$NHCH_2$), 30.12 (–$NHCH_2CH_2$), 29.74 [2×–$N(CH_2)_2CH_2$], 27.54 [–$N(CH_2)_3CH_2$], 27.17 and 26.65 [2 × >C(CH_3)$_2$]. Anal. calcd for $C_{25}H_{46}N_2O_8$: C, 59.76; H, 9.16; N, 5.57; Found: C, 57.44; H, 8.93; N, 4.27.

***N*¹,*N*¹⁰-Bis-[5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,10-diaminodecane (5).** Reaction of 3-*O*-methyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-ulose (**2a**, 1.0 g, 4.9 mmol), 1,10-diaminodecane (0.43 g, 2.50 mmol), Et_3N (1.5 mL) and $NaBH_4$ (0.10 g, 2.63 mmol) in absolute ethanol as above gave **5** as colourless oil. Yield 70%; $[\alpha]_D = -51.63$ (*c* 0.14, $CHCl_3$); MS (FAB): *m/z* = 545 $(M+H)^+$; IR (KBr): ν cm^{-1} 3141 (–NH), 2980–2800 (CH_3 and CH_2 stretching); ^1H NMR (200 MHz, $CDCl_3$): δ 5.89 (d, *J* = 3.84 Hz, 1H, H-1), 4.58 (d, *J* = 3.84 Hz, 1H, H-2), 4.30 (m, 1H, H-4), 3.69 (d, *J* = 3.1 Hz, 1H, H-3), 3.41(OCH_3), 2.90 (m, 2H, H-5), 2.67 (t, *J* = 6.87 Hz, 4H, 2 × –NCH_2), 1.90 (bs, 1H, –NH), 1.49 and 1.32 [each s, each 3H, 2× >C(CH_3)$_2$], 1.27 (m, *J* = 16 Hz, CH_2's); ^{13}CNMR ($CDCl_3$): δ 112.06 [>C(CH_3)$_2$], 105.20 (C-1), 85.04 (C-2), 82.03 (C-3), 80.03 (C-4), 58.14 (OCH_3), 50.61 (C-5), 48.35 (–$NHCH_2$), 30.32 (–$NHCH_2CH_2$), 30.05 [2× $N(CH_2)_2CH_2$], 29.89 [2× –$N(CH_2)_3CH_2$], 27.66 [2×–$N(CH_2)_4CH_2$], 27.11 and 26.65 [2 × >C(CH_3)$_2$]. Anal. calcd for $C_{28}H_{52}N_2O_8$: C, 61.76; H, 9.56; N, 5.15; Found: C, 59.72; H, 10.01; N, 4.96.

***N*¹,*N*¹²-Bis-[5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,12-diaminododecane (6).** Reaction of 3-*O*-methyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-ulose (**2a**, 1.0 g, 4.9 mmol), 1,12-diaminododecane (0.5 g, 2.50 mmol), Et_3N (1.5 mL) and $NaBH_4$ (0.10 g, 2.63 mmol) in absolute ethanol as above afforded **6** as colourless oil. Yield 70%; $[\alpha]_D = -74.52$ (*c* 0.24, $CHCl_3$); MS (FAB): *m/z* = 573 $(M+H)^+$; IR (KBr): ν cm^{-1} 3020 (–NH), 2929 and 2856 (CH_3 and CH_2 stretching): ^1H NMR (200 MHz, $CDCl_3$): δ 5.90 (d, *J* = 3.8 Hz, 1H, H-1), 4.58 (d, *J* = 3.8 Hz, 1H, H-2), 4.32 (m, 1H, H-4), 3.71 (d, *J* = 3.0 Hz, 1H, H-3), 3.44 (OCH_3), 2.92 (m, 2H, H-5), 2.67 (t, *J* = 6.9 Hz, 4H, 2 × –NCH_2), 1.91(bs, 1H, –NH), 1.52 and 1.32 [each s, each 3H, 2× >C(CH_3)$_2$], 1.31 (m, 10H, CH_2's); ^{13}C NMR ($CDCl_3$): δ 112.78 [>C(CH_3)$_2$], 105.23 (C-1), 84.41 (C-2), 81.94 (C-3), 76.89 (C-4), 57.66 (OCH_3), 53.84 (C-5), 49.36 (–$NHCH_2$), 47.06 (–$NHCH_2CH_2$), 29.75 [2× –$N(CH_2)_2CH_2$], 29.57 [2×–$N(CH_2)_3CH_2$], 27.88 [2× –$N(CH_2)_4CH_2$], 27.16 [2×–$N(CH_2)_5CH_2$], 27.05 and 26.49 [2 × >C(CH_3)$_2$]. Anal. calcd for $C_{30}H_{56}N_2O_8$: C, 62.94; H, 9.79; N, 4.89; Found: C, 59.14; H, 9.75; N, 4.59.

***N*¹,*N*⁷-Bis-[3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,7-diaminoheptane (7).** Reaction of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-ulose (**2b**, 3.3 g, 11.67 mmol), 1,7-diaminoheptane (0.77 g, 5.93 mmol), Et_3N (3.8 mL) and $NaBH_4$ (0.23 g, 6.05 mmol) in absolute ethanol as above gave **7** as colourless oil. Yield 65%, $[\alpha]_D = -60.6$ (*c* 0.13, $CHCl_3$); MS (FAB): *m/z* = 655 $(M+H)^+$; IR (KBr): ν cm^{-1} 3141 (–NH), 2980–2800 (CH_3 and CH_2 stretching), 1631 (C=C); ^1H NMR (200 MHz, $CDCl_3$): δ 7.32 (m, 10H, Ar–H), 5.91 (d, *J* = 3.8 Hz, 1H, H-1), 4.68 (d, *J* = 11.7 Hz, 1H, –OCH_APh), 4.60 (d, *J* = 3.8 Hz, 1H, H-2), 4.49 (d, *J* = 11.9 Hz, 1H, –OCH_BPh), 4.42 (m, 1H, H-4), 3.95 (d, *J* = 3.1 Hz, 1H, H-3), 3.02 (m, 2H, H-5), 2.68 (m, 4H, 2×–NCH_2), 1.90 (bs, 1H, –NH), 1.48 and 1.30 [each s, each 3H, 2× >C(CH_3)$_2$], 1.30 (m, 10H, CH_2's); ^{13}C NMR ($CDCl_3$): δ 137.78, 128.9, 128.39 and 128.11 (Ar–C), 112.09 [>C(CH_3)$_2$], 105.35 (C-1), 82.56 (C-2), 82.51 (C-3), 79.15 (C-4), 72.18 (–OCH_2Ph), 50.11 (C-5), 47.99 (–$NHCH_2$), 29.56 (–$NHCH_2CH_2$), 29.37 [2× –$N(CH_2)_2CH_2$], 27.37 [2 ×–$N(CH_2)_3CH_2$], 27.18 and 26.72 [2 × >C(CH_3)$_2$]. Anal. calcd for $C_{37}H_{54}N_2O_8$: C, 67.89; H, 8.26; N, 4.28; Found: C, 68.81; H, 7.96; N, 4.12.

***N*¹,*N*¹⁰-Bis-[3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,10-diaminodecane (8).** Reaction of 3-*O*-benzyl-1, 2-*O*-isopropylidene-α-D-xylofuranos-5-ulose (**2b**, 1.21 g, 4.35 mmol), 1,10-diaminodecane (0.378 g, 2.1977 mmol), Et_3N (1.7 mL) and $NaBH_4$ (0.1 g, 2.66 mmol) in absolute ethanol as above gave **8** as colourless oil. Yield 70%; $[\alpha]_D = -48.53$ (*c* 0.18, $CHCl_3$); MS (FAB): *m/z* = 697 $(M+H)^+$; IR (KBr): ν cm^{-1} 3118 (–NH), 2931 and 2860 (CH_3 and CH_2 stretching); ^1H NMR (200 MHz, $CDCl_3$): δ 7.32 (m, 10H, Ar–H), 5.92 (d, *J* = 3.7 Hz, 1H, H-1), 4.69 (d, *J* = 11.8 Hz, 1H, –OCH_APh), 4.62 (d, *J* = 3.7 Hz, 1H, H-2), 4.48 (d, *J* = 11.8 Hz, 1H, –OCH_BPh), 4.43 (m, 1H, H-4), 3.93 (d, *J* = 3.0 Hz, 1H, H-3), 2.94 (m, 2H, H-5), 2.63 (m, 4H, 2 ×–NCH_2), 1.91(s, 1H, –NH), 1.48 and 1.31 [each s, each 3H, 2× >C(CH_3)$_2$], 1.30 (m, 8H, CH_2's); ^{13}CNMR ($CDCl_3$): δ 137.81, 129.17, 128.95 and 128.44 (Ar–C), 112.07 [>C(CH_3)$_2$], 105.26 (C-1), 83.05 (C-2), 82.45 (C-3), 79.5 (C-4), 72.39 (–OCH_2Ph), 50.61 (C-5), 48.25 (–$NHCH_2$), 29.83, 29.75, 29.54 and 27.43 (CH_2's), 27.18 and 26.94 [2 × >C(CH_3)$_2$]. Anal. calcd for $C_{40}H_{66}N_2O_8$: C, 68.96; H, 8.62; N, 4.03; Found: C, 67.98; H, 8.76; N, 3.93.

***N*¹,*N*¹²-Bis-[3-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,12-diaminododecane (9).** Reaction of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranos-5-ulose (**2b**, 1.13 g, 4.06 mmol), 1,12-diaminododecane (0.41 g, 2.05 mmol), Et_3N (1.6 mL) and $NaBH_4$ (0.09 g, 2.36 mmol) in absolute ethanol as above gave **9** as colorless oil. Yield 70%; $[\alpha]_D = -50.66$ (*c* 0.22, $CHCl_3$); MS (FAB): *m/z* = 725 $(M+H)^+$; IR (KBr): ν cm^{-1} 3020 (–NH), 2929 and 2856.4 (CH_3 and CH_2 stretching), 1519.8 (C=C); ^1H NMR (200 MHz, $CDCl_3$): δ 7.32 (m, 10H, Ar–H), 5.92 (d, *J* = 3.8 Hz, 1H, H-1), 4.69 (d, *J* = 12.6 Hz, 1H, –OCH_APh), 4.61 (d, *J* = 3.8 Hz, 1H, H-2), 4.47 (d, *J* = 12.0 Hz, 1H, –OCH_BPh), 4.31 (m, 1H, H-4), 3.89 (d, *J* = 3.1 Hz, 1H, H-3), 2.93 (m, 2H, H-5), 2.59

(t, $J=6.9$ Hz, 3H, 2 ×–NCH_2), 1.85 (bs, 1H, –NH), 1.47 and 1.31 [each s, each 3H, 2× >C(CH_3)$_2$], 1.30 (m, 10H, CH_2's); ^{13}C NMR ($CDCl_3$): δ 137.89, 128.89, 128.36 and 128.09 (Ar–C), 111.93 [>C(CH_3)$_2$], 105.29 (C-1), 82.75 (C-2), 82.37 (C-3), 79.99 (C-4), 72.14 (–OCH_2Ph), 53.84 (C-5), 50.61, 48.45, 30.34, 29.95 and 27.68 (CH_2's), 27.18 and 26.72 [2 × >C (CH_3)$_2$]. Anal. calcd for $C_{42}H_{64}N_2O_8$: C, 69.61; H, 8.84; N, 3.87; Found: C, 68.12; H, 9.10; N, 3.82.

General procedure

(5*R*, 5*S*) N^1,N^3-bis-[5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,3-diaminopropane (11a) and (5*S*, 5*S*) N^1,N^3-bis-[5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,3-diaminopropane (11b). A solution of (E) ethyl-(3-*O*-methyl-5, 6-dideoxy-1, 2-*O*-isopropylidene)-α-D-1,4-heptofuranosyl-5-enoate (**10a**, 1.38 g, 5.07 mmol) and 1,3-diamino propane (0.22 mL, 2.56 mmol) in ethanol (10 mL) was magnetically stirred at ambient temperature for 18 h. The solvent evaporated from the reaction mixture under reduced pressure and the crude product was extracted with chloroform (2 × 50 mL), washed with water (10 mL), dried (Na_2SO_4) and evaporated under reduced pressure. The crude product, a diastereoisomeric mixture (6:45:49) of three compounds on column chromatography over SiO_2 using chloroform: methanol (98:2) as eluant gave **11a** and **11b** in pure forms. Yield 82%; **11a** MS (FAB): $m/z=619$ $(M+H)^+$; $[\alpha]_D=-50.40$ (*c* 0.25, $CHCl_3$); IR (KBr): ν cm^{-1} 1725 (>C=O), 2870 and 2950 (CH_3 and CH_2 stretching), 3020 (–NH); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.89 (d, $J=3.8$ Hz, 1H, H-1), 5.84 (d, $J=3.8$ Hz, 1H, H-1′), 4.58 (d, $J=3.8$ Hz, 1H, H-2), 4.54 (d, $J=3.8$ Hz, 1H, H-2′), 4.19–4.01 (m, 6H, H-4, H-4′, OCH_2 and O$CH_{2'}$), 3.80(d, $J=3.0$ Hz, 1H, H-3), 3.68 (d, $J=3.0$ Hz, 1H, H-3), 3.42–3.35 (m, 8H, H-5, H-5′, OCH_3 and OCH_3'), 2.78–2.42 (m, 8H, H-6 and NHCH_2), 1.63 and 1.60 (bs, 2H, –NH), 1.47 and 1.31 [each s, each 3H, 2 × >C(CH_3)$_2$], 1.27(m, 8H, CH_2 and 2 ×–OCH_2CH_3). **11b** colourless oil. MS (FAB) *m*/*z* 619 $(M+H)^+$; $[\alpha]_D=-58.66$ (*c* 0.26, $CHCl_3$); IR (KBr): ν cm^{-1} 3020 (–NH), 2941 and 2842 (CH_3 and CH_2 stretching), 1728 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.89 (d, $J=3.8$ Hz, 1H, H-1), 4.57 (d, $J=3.8$ Hz, 1H, H-2), 4.18–4.07 (m, 3H, H-4 and –O$CH_2$$CH_3$), 3.70 (d, $J=3.0$ Hz, 1H, H-3), 3.41 (m, 1H, H-5), 3.36 (s, 3H–OCH_3), 2.70 (m, 4H, 2 × –NCH_2), 2.42 (m, 2H, H-6), 1.70 (bs, 1H, –NH), 1.49 and 1.31 [each s, each 3H, 2 × >C(CH_3)$_2$], 1.25 (m, 5H, CH_2 and OCH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 172.0 (>C=O), 112.08 [>C(CH_3)$_2$], 105.15 (C-1), 84.38 (C-2), 82.26 (C-4), 81.51(C-3), 60.91 (–O$CH_2$$CH_3$), 57.60 (–$OCH_3$), 54.58 (C-5), 46.68 ($NCH_2$), 36.26 (C-6), 29.57 ($CH_2$), 27.12 and 26.69 [2 × >C (CH_3)$_2$], 14.57 (–OCH_2CH_3). Anal. calcd for $C_{29}H_{50}N_2O_{12}$: C, 56.31; H, 8.09; N, 4.53; Found: C, 55.92; H, 8.14; N, 4.25.

N^1,N^7-Bis-[5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranosyl]-1,7-diaminoheptane (12). It was obtained by reaction of ethyl-(-5,6-dideoxy-1,2-*O*-isopropylidene)-3-*O*-methyl-α-D-1,4-heptofuranosyl-5-enoate (**10a**, 3.56 g, 13.08 mmol), 1,7-diamino heptane (0.86 g, 6.615 mmol) as described above and isolated as diastereomeric mixture (45:50) as colourless oil. Yield 85%; $[\alpha]_D=-53.86$ (*c* 0.18, $CHCl_3$); MS (FAB): $m/z=675$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1732 (>C=O), 2862 and 2941 (CH_3 and CH_2 stretching), 3022 (–NH); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.90 and 5.85 (each d, $J=3.8$ Hz, each 1H, diastereomeric H-1), 4.58 and 4.55 (each d, $J=3.8$ Hz, each 1H, diastereomeric H-2), 4.18 (m, 1H, H-4), 4.11 (q, $J=7.1$ Hz, 2H, –O$CH_2$$CH_3$), 3.85 and 3.72 (each d, $J=3.0$ Hz, each 1H, diastereomeric H-3), 3.40 and 3.37 (s, 3H –OCH_3), 3.2 (m, 1H, H-5), 2.68 (m, 2H, H-6), 2.49 (m, 4H, 2 ×–NCH_2), 1.96 (bs, 1H, –NH), 1.48 and 1.32 [each s, each 3H, 2× >C(CH_3)$_2$], 1.29 (m, 10H, CH_2's), 1.23 (t, $J=7.1$ Hz, 3H, –O$CH_2$$CH_3$); ^{13}C NMR ($CDCl_3$): δ 173.28 and 172.62 (diastereomeric >C=O), 111.91 and 111.84 [diastereomeric >C(CH_3)$_2$], 105.11 and 105.07 (diastereomeric C-1), 84.46 and 84.20 (diastereomeric C-2), 82.59 and 82.30 (diastereomeric C-4), 81.89 and 81.57 (diastereomeric C-3), 60.74 and 60.59 (–O$CH_2$$CH_3$), 58.02 and 57.59 (–$OCH_3$), 54.62 and 52.89 (diastereomeric C-5), 47.64 and 47.54 (2 × –NCH_2), 36.74 and 36.45 (diastereomeric C-6), 30.91, 30.62, 29.83, 27.65 and 27.15(CH_2's), 27.12 and 26.69 [2 × >C(CH_3)$_2$], 14.60 (–OCH_2CH_3). Anal. calcd for $C_{33}H_{50}N_2O_{12}$: C, 58.74; H, 8.61; N, 4.15; Found: C, 57.98; H, 8.67; N, 4.20.

N^1,N^{10}-Bis-[5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,10-diaminodecane (13). It was obtained by reaction of ethyl-(5,6-dideoxy-1,2-*O*-isopropylidene-3-*O*-methyl)-α-D-1,4-heptofuranosyl-5-enoate (**10a**, 1.9 g, 6.98 mmol), 1,10-diamino decane (0.61 g, 3.54 mmol) as described above and isolated as diastereomeric mixture (45:50) colourless oil. Yield 90%; $[\alpha]_D=-70.0$ (*c* 0.12, $CHCl_3$); MS (FAB): $m/z=717$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1730 (>C=O), 2864 and 2933 (CH_3 and CH_2 stretching), 3022 (–NH); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.90 and 5.85 (each d, $J=3.8$ Hz, each 1H, diastereomeric H-1), 4.58 and 4.55 (d, $J=3.8$ Hz, each 1H, diastereomeric H-2), 4.18–4.11 (m, 3H, –O$CH_2$$CH_3$ and H-4), 3.72 and 3.67 (d, $J=2.8$ Hz, each 1H, diastereomeric H-3), 3.41 and 3.37 (s, 3H, –OCH_3), 3.31 (m, 1H, H-5), 2.63 (m, 2H, H-6), 2.45 (m, 4H, 2 × –NCH_2), 1.62 (bs, 1H, –NH), 1.48 and 1.31 [each s, each 3H, 2× >C(CH_3)$_2$], 1.25 (m, 19H, CH_2's and –OCH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 173.50 and 172.84 (>C=O), 112.09 [>C(CH_3)$_2$], 105.32 (C-1), 84.63 and 84.39 (diastereomeric C-2), 82.84 and 82.56 (diastereomeric C-4), 82.08 and 81.76 (diastereomeric C-3), 60.93 and 60.79 (–O$CH_2$$CH_3$), 58.21 and 57.79 (–$OCH_3$), 54.84 and 53.10 (diastereomeric C-5), 47.83 and 47.2 (2 × –NCH_2), 36.97 and 36.79 (diastereomeric C-6), 31.17, 30.88, 30.14 and 27.89 (CH_2's), 27.35 and 26.91 [2 × >C(CH_3)$_2$], 14.82 (–OCH_2CH_3). Anal. calcd for $C_{36}H_{64}N_2O_{12}$: C, 66.36; H, 8.2; N, 3.23; Found: C, 65.86; H, 8.44; N, 3.18.

N^1,N^{12}-Bis-[5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-xylofuranos-5-yl]-1,12-diaminododecane (14). It was obtained by reaction of ethyl-(5, 6-dideoxy-1,2-*O*-isopropylidene-3-*O*-methyl)-α-D-1,4-hep-

tofuranosyl-5-enoate (**10a**, 1.0 g, 3.67 mmol), 1,12-diamino dodecane (0.37 g, 1.84 mmol) as described above and isolated as diastereomeric mixture (46:50). Colourless oil. Yield 92%. $[\alpha]_D = -45.56$ (*c* 0.28, $CHCl_3$); MS (FAB): $m/z = 745$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1727 (>C=O), 2855 and 2929 (CH_3 and CH_2 stretching), 3342 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 5.90 (d, $J = 3.7$ Hz, 1H, H-1), 4.58 (d, $J = 3.7$ Hz, 1H, H-2), 4.19–4.12 (m, 3H, $-OCH_2CH_3$ and H-4), 3.72 (d, $J = 3.2$ Hz, 1H, H-3), 3.37 (s, 3H, $-OCH_3$), 3.35 (m, 1H, H-5), 2.62 (m, 2H, H-6), 2.45 (m, 4H, 2 × $-NCH_2$), 1.95 (bs, 1H, –NH), 1.48 and 1.31 [each s, each 3H, 2× $>C(CH_3)_2$], 1.26 (m, 23H, CH_2's and $-OCH_2CH_3$); ^{13}CNMR ($CDCl_3$): δ 172.17 (>C=O), 111.86 [$>C(CH_3)_2$], 105.09 (C-1), 84.43 (C-2), 82.35 (C-4), 81.57 (C-3), 60.72 ($-OCH_2CH_3$), 57.58 ($-OCH_3$), 54.61 (C-5), 47.65 (2× $-NCH_2$), 36.76 (C-6), 30.99, 30.68, 29.97 and 27.69 (CH_2's), 27.14 and 26.68 [2 × >C ($CH_3)_2$], 14.59 ($-OCH_2CH_3$). Anal. calcd for $C_{38}H_{68}N_2O_{12}$: C, 61.29; H, 9.14; N, 3.76; Found: C, 69.36; H, 9.68; N, 3.94.

***(5R,5S) N^1,N^3*-Bis-[3-*O*-benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,3-diaminopropane (15a) and *(5S,5S) N^1,N^3*-bis-[3-*O*-benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,3-diaminopropane (15b).** These were obtained by reaction of ethyl-(3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene)-α-D-1,4-heptofuranosyl-5-enoate (**10b**, 1 g, 2.87 mmol), 1,3-diaminopropane (0.12 mL, 1.62 mmol) as described above and isolated as diastereomeric mixture in (7:12) ratio Yield 85%. **15a** Colourless oil $[\alpha]_D = -63.11$ (*c* 0.11, $CHCl_3$); MS (FAB): $m/z = 771$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1722 (>C=O), 2860 and 2945 (CH_3 and CH_2 stretching), 3022 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.33 (m, 10H, Ar–H), 5.93 (d, $J = 3.8$ Hz, 1H, H-1), 5.87 (d, $J = 3.8$ Hz, 1H, H-1′), 4.71–4.52 (m, 5H, OCH_2Ph, H-2, H-2′ and $OCH_{B'}Ph$), 4.42 (d, $J = 11.7$ Hz, 1H, $-OCH_BPh$), 4.20–4.02 (m, 7H, H-4, H-4′, OCH_2, $OCH_{2'}$ and H-3′), 3.91 (d, $J = 3.0$ Hz, 1H, H-3), 3.42–3.35 (m, 2H, H-5 and H-5′), 2.77–2.29 (m, 8H, H-6, H-6′ and $NHCH_2$), 1.60 and 1.53 (bs, 2H, –NH), 1.48, 1.47, 1.32 and 1.31 [each s, each 3H, 2 × $>C(CH_3)_2$], 1.27 (m, 8H, CH_2 and 2×$-OCH_2CH_3$). **15b** Colourless oil $[\alpha]_D = -48.61$ (*c* 0.16, $CHCl_3$); MS (FAB): $m/z = 771$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1720 (>C=O), 2856 and 2931 (CH_3 and CH_2 stretching), 3020 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.32 (m, 10H, Ar–H), 5.90 (d, $J = 3.9$ Hz, 1H, H-1), 4.68 (d, $J = 11.8$ Hz, 1H, $-OCH_APh$), 4.62 (d, $J = 3.9$ Hz, 1H, H-2), 4.44 (d, $J = 11.8$ Hz, 1H, $-OCH_BPh$), 4.22 and 4.18 (dd, $J = 3.0$ Hz and 8.8 Hz, 1H, H-4), 4.07 (q, $J = 7.1$ Hz, 2H, $-OCH_2CH_3$), 3.92 (d, $J = 3.0$ Hz, 1H, H-3), 3.42 (m, 1H, H-5), 2.71 (t, $J = 6.7$ Hz, 2H, $NHCH_2$), 2.34 (d, $J = 5.4$ Hz, 1H, H-6), 1.60 (bs, 1H, –NH), 1.48 and 1.31 [each s, each 3H, 2 × $>C(CH_3)_2$], 1.25 (m, 8H, NCH_2CH_2 and 2× $-OCH_2CH_3$); ^{13}C NMR ($CDCl_3$): δ 171.87 (>C=O), 137.24, 128.92, 128.54 and 128.37 (Ar–C), 112.38 [$>C(CH_3)_2$], 105.24 (C-1), 82.09 (C-2), 81.94 (C-4), 81.78 (C-3), 71.96 ($-OCH_2Ph$), 60.06 ($-OCH_2CH_3$), 54.39 (C-5), 47.60 (2 × $-NCH_2$), 35.55 (C-6), 30.80 (NCH_2CH_2), 27.19 and 26.77 [2 × $>C(CH_3)_2$], 14.51 ($-OCH_2CH_3$). Anal. calcd for $C_{41}H_{58}N_2O_{12}$: C, 63.89; H, 7.53; N, 3.64; Found: C, 62.93; H, 7.84; N, 3.75.

***(5R,5S) N^1,N^7*-Bis-[3-*O*-benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-α-D-gluco and β-L-ido-1,4-pentofuranosyl]-1,7-diaminoheptane (16a) and *(5S,5S) N^1,N^7*-bis-[3-*O*_benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-β-L-ido-1,4-pentofuranosyl]-1,7-diaminoheptane (16b).** These were obtained by reaction of ethyl-(3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene)-α-D-1,4-heptofuranosyl-5-enoate (**10b**, 2.36 g, 6.78 mmol), 1,7-diaminoheptane (0.45 g, 3.46 mmol) as described above and isolated as diastereoisomeric mixture (36:60). Colourless oil, Yield 90%. **16a** Colourless oil $[\alpha]_D = -46.6$ (*c* 0.07, $CHCl_3$); MS (FAB): $m/z = 827$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1722 (>C=O), 2866 and 2941 (CH_3 and CH_2 stretching), 3020 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.33 (m, 10H, Ar–H), 5.94 (d, $J = 3.8$ Hz, 1H, H-1), 5.88 (d, $J = 3.7$ Hz, 1H, H-1′), 4.69 (d, $J = 11.8$ Hz, 1H, $-OCH_APh$), 4.65–4.51 (m, 4H, $OCH_{A'}$, H-2, H-2′ and OCH_BPh), 4.42 (d, $J = 11.7$ Hz, 1H, $-OCH_BPh$), 4.24–4.07 (m, 6H, H-4, H-4′, OCH_2 and $OCH_{2'}$), 4.04 (d, $J = 2.8$ Hz, 1H, H-3′), 3.93 (d, $J = 3.0$ Hz, 1H, H-3), 3.45–3.35 (m, 2H, H-5 and H-5′), 2.81–2.20 (m, 8H, H-6 and $NHCH_2$), 1.60 and 1.53 (bs, 2H, –NH), 1.48, 1.47, 1.32 and 1.31 [each s, each 3H, 2 × $>C(CH_3)_2$], 1.29–1.19 (m, 16H, CH'_2s and $-OCH_2CH_3$); ^{13}C NMR ($CDCl_3$): δ 172.87 and 172.20 (>C=O), 138.09, 137.50, 128.85, 128.41, 128.26, 128.24 and 128.02 (Ar–C), 111.95 and 111.83 [$>C(CH_3)_2$], 105.19 and 105.05 (C-1 and C-1′), 82.46 and 82.22 (C-2 and C-2′), 82.21 and 82.13 (C-4 and C-4′), 82.08 and 81.89 (C-3 and C-3′), 71.99 and 71.85 ($-OCH_2Ph$), 60.70 and 60.58 ($-OCH_2CH_3$), 54.41 and 52.90 (C-5 and C-5′), 47.69 and 47.40 (NCH_2 and $NCH_{2'}$), 36.72 and 36.23 (C-6 and C-6′), 30.85, 30.70, 29.87 and 27.67 (CH_2's), 27.19, 27.12, 26.75 & 26.71 [$>C(CH_3)_2$], 14.59 and 14.55 ($-OCH_2CH_3$). **16b**. $[\alpha]_D = -76.8$ (*c* 0.06, $CHCl_3$); MS (FAB): $m/z = 827$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1726 (>C=O), 2862 and 2933 (CH_3 and CH_2 stretching), 3022 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.33 (m, 10H, Ar–H), 5.93(d, $J = 3.9$ Hz, 1H, H-1), 4.69 (d, $J = 11.8$ Hz, 1H, $-OCH_APh$), 4.63 (d, $J = 3.9$ Hz, 1H, H-2), 4.44 (d, $J = 11.8$ Hz, 1H, $-OCH_BPh$), 4.20 and 4.15 (dd, $J = 3.0$ Hz and 8.8 Hz, 1H, H-4), 4.09 (q, $J = 7.1$ Hz, 2H, $-OCH_2CH_3$), 3.93 (d, $J = 3.0$ Hz, 1H, H-3), 3.42 (m, 1H, H-5), 2.60 (m, 2H, $NHCH_2$), 2.42 and 2.34 (dd, $J = 4.7$ Hz and 15.0 Hz, 1H, $H\text{-}6_A$), 2.29 and 2.22 (dd, $J = 6.4$ Hz and 15.0 Hz, 1H, $H\text{-}6_B$), 1.96 (bs, 1H, –NH), 1.48 and 1.31 [each s, each 3H, 2 × $>C(CH_3)_2$], 1.25 (m, 8H, CH_2's and $-OCH_2CH_3$), ^{13}C NMR ($CDCl_3$): δ 172.20 (>C=O), 137.49, 128.85, 128.40 and 128.02 (Ar–C), 111.95 [$>C(CH_3)_2$], 105.18 (C-1), 82.45 (C-2), 82.13 (C-4), 81.89 (C-3), 71.85 ($-OCH_2Ph$), 60.69 ($-OCH_2CH_3$), 54.41 (C-5), 47.69 (2 × $-NCH_2$), 36.71 (C-6), 30.94, 29.85 and 27.67(CH_2's), 27.12 and 26.70 [2 × >C ($CH_3)_2$], 14.55 ($-OCH_2CH_3$). Anal. calcd for $C_{45}H_{64}N_2O_{12}$: C, 65.37; H, 7.99; N, 3.39; Found: C, 64.83; H, 8.32; N, 3.95.

***(5R,5S) N^1,N^{10}*-bis- [3-*O*-benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,10-diaminodecane (17a) and *(5S,5S) N^1,N^{10}*-bis-[3-*O*-benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,10-diaminodecane (17b).** These were obtained by reaction of ethyl-(3-*O*-benzyl-5,6-dideoxy-

1,2-*O*-isopropylidene)-α-D-1,4-heptofuranosyl-5-enoate (**10b**, 2.26 g, 6.49 mmol), 1,10-diaminodecane (0.61 g, 3.54 mmol) as described above as diastereoisomeric mixture (35:61 ratio), Yield 90%. **17a** colourless oil, $[\alpha]_D = -40.42$ (*c* 0.24, $CHCl_3$); MS (FAB): $m/z = 869$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1721 (>C=O), 2858 and 2943 (CH_3 and CH_2 stretching), 3023 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.33 (m, 10H, Ar–H), 5.94 (d, $J = 3.8$ Hz, 1H, H-1), 5.88 (d, $J = 3.8$ Hz, 1H, H-1′), 4.69 (d,$J = 11.8$ Hz, 1H, –OC$\underline{H}_A$Ph), 4.64–4.59 (m, 4H, OC$\underline{H}_{A'}$, H-2, H-2′ and OC$\underline{H}_B$Ph), 4.44 (d, $J = 11.8$ Hz, 1H, –OC$\underline{H}_B$Ph), 4.21–4.07 (m, 6H, H-4, H-4′, OC$\underline{H}_2$ and OC$\underline{H}_{2'}$), 4.04 (d, $J = 2.9$ Hz, 1H, H-3′), 3.93 (d, $J = 3.1$ Hz, 1H, H-3), 3.46–3.35 (m, 2H, H-5 and H-5′), 2.82–2.20 (m, 8H, H-6, H-6′ and 2 × NHC$\underline{H}_2$), 1.54 and 1.53 (bs, 2H, –NH), 1.48, 1.47, 1.32 and 1.31 [each s, each 3H, 2 × >C(C$\underline{H}_3$)$_2$], 1.28–1.19 (m, 22H, CH_2's and –OCH$_2$C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 172.91 and 172.22 (>C=O), 138.04, 137.53, 128.88, 128.85, 128.43, 128.27 and 128.12 (Ar–C), 111.99 and 111.92 [>C(CH$_3$)$_2$], 105.23 and 105.15 (C-1 and C-1′), 82.65 and 82.52 (C-2 and C-2′), 82.31 and 82.22 (C-4 and C-4′), 82.12 and 82.07 (C-3 and C-3′), 72.44 and 71.89 (–OCH$_2$Ph), 60.71 and 60.57 (–OCH$_2$CH$_3$), 54.48 and 53.09 (C-5 and C-5′), 47.77 and 47.40 (NCH_2 and $NCH_{2'}$), 36.75 and 36.23 (C-6 and C-6′), 31.12, 30.83, 29.99 and 27.76 (CH_2's), 27.21, 27.15, 26.77 and 26.73 [>C(CH$_3$)$_2$], 14.63 and 14.59 (–OCH$_2$CH$_3$). **17b** colourless solid mp = 94 °C; $[\alpha]_D = -28.09$ (*c* 0.26, $CHCl_3$); (FAB MS): $m/z = 869$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1720 (>C=O), 2851 and 2933 (CH_3 and CH_2 stretching), 3016 (–NH); ^{1}HNMR (200 MHz, $CDCl_3$): δ 7.32 (m, 10H, Ar–H), 5.94 (d,$J = 3.9$ Hz, 1H, H-1), 4.69 (d, $J = 11.8$ Hz, 1H, –OC$\underline{H}_A$Ph), 4.63(d, $J = 3.9$ Hz, 1H, H-2), 4.44 (d, $J = 11.8$ Hz, 1H, –OC$\underline{H}_B$Ph), 4.20 and 4.16 (dd, $J = 3.1$ Hz and 8.8 Hz, 1H, H-4), 4.09 (q, $J = 7.1$ Hz, 2H, –OC$\underline{H}_2$CH$_3$), 3.93 (d, $J = 3.1$ Hz, 1H, H-3), 3.41 (m, 1H, H-5), 2.61 (m, 2H, NHC$\underline{H}_2$), 2.42 and 2.35 (dd, $J = 4.8$ Hz and 14.8 Hz, 1H, H-6$_A$), 2.30 and 2.23 (dd, $J = 6.4$ Hz and 14.8 Hz, 1H, H-6$_B$), 1.67 (bs, 1H, –NH), 1.48 and 1.31 [each s, each 3H, 2 × >C(C$\underline{H}_3$)$_2$], 1.26 (m, 11H, CH_2's and –OCH$_2$C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 172.21 (>C=O), 137.53, 128.86, 128.41 and 128.26 (Ar–C), 111.97 [>C(CH$_3$)$_2$], 105.22 (C-1), 82.48 (C-2), 82.25 (C-4), 82.19 (C-3), 71.89 (–OCH$_2$Ph), 60.69 (–OCH$_2$CH$_3$), 54.46 (C-5), 47.74 (2 × –NCH$_2$), 36.75 (C-6), 30.79, 29.67, 27.75 and 27.73 (CH_2's), 27.15 and 26.73 [2 × >C(CH$_3$)$_2$], 14.58 (–OCH$_2$CH$_3$). Anal. calcd for $C_{48}H_{72}N_2O_{12}$: C, 60.34; H, 8.94; N, 3.91; Found: C, 59.83; H, 8.97; N,4.14.

(5*R*,5*S*)*N*^{1},*N*^{12}-Bis-[3-*O*-benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylo furanos-5-yl]-1,12-diaminododecane (18a) and (5*S*,5*S*) *N*^{1},*N*^{12}-bis-[3-*O*-benzyl-5-carbethoxymethyl-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl]-1,12-diaminododecane (18b). These were obtained by reaction of ethyl- (3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene)-α-D-1,4-heptofuranosyl-5-enoate (**10b**, 3.35 g, 9.6 mmol) in ethanol (35 mL), 1,12-diaminododecane (0.96 g, 4.81 mmol) as described above as diastereoisomeric mixture (35:61 ratio), Yield 92%. **18a** Colouress oil $[\alpha]_D = -45.45$ (*c* 0.27, $CHCl_3$); MS (FAB): $m/z = 897$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1720 (>C=O), 2860 and 2941 (CH_3 and CH_2 stretching), 3025 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.33 (m, 10H, Ar–H), 5.93 (d, $J = 3.8$ Hz, 1H, H-1), 5.88 (d, $J = 3.8$ Hz, 1H, H-1′), 4.69 (d, $J = 11.8$ Hz, 1H, –OC$\underline{H}_A$Ph), 4.64–4.58 (m, 4H, OC$\underline{H}_{A'}$, H-2, H-2′ and OC$\underline{H}_{B'}$Ph), 4.44 (d, $J = 11.8$ Hz, 1H, –OC$\underline{H}_B$Ph), 4.21–4.07 (m, 6H, H-4, H-4′, OC$\underline{H}_2$ and OC$\underline{H}_{2'}$), 4.04 (d, $J = 3.0$ Hz, 1H, H-3′), 3.93 (d, $J = 3.1$ Hz, 1H, H-3), 3.40 (m, 2H, H-5 and H-5′), 2.82–2.29 (m, 8H, H-6, H-6′ and 2 × NHC$\underline{H}_2$), 1.61 and 1.60 (bs, 2H, –NH), 1.48, 1.47, 1.32 and 1.31 [each s, each 3H, 2 × >C(C$\underline{H}_3$)$_2$], 1.28–1.20 (m, 26H, CH_2's and –OCH$_2$C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 172.23 and 172.20 (>C=O), 138.04, 137.52, 128.87, 128.84, 128.42, 128.27 and 128.12 (Ar–C), 112.00 and 111.92 [>C(CH$_3$)$_2$], 105.44 (C-1), 105.23 (C-1′), 82.67 (C-2), 82.47 (C-2′), 82.29 (C-4), 82.25 (C-4′), 82.18 (C-3), 82.13 (C-3′), 72.45, 71.91 (–OCH$_2$Ph), 60.71 and 60.56 (–OCH$_2$CH$_3$), 54.48, 53.11 (C-5 and C-5′), 47.76 and 47.39 (NCH_2 and $NCH_{2'}$), 36.70 and 36.22 (C-6 and C-6′), 31.08, 30.77, 30.02, 27.74 ($CH'_{2\ s}$), 27.21, 27.15, 26.77 and 26.73 [>C(CH$_3$)$_2$], 14.59 and 14.58 (–OCH$_2$CH$_3$). **18b**. Colourless solid, mp = 75 °C; $[\alpha]_D = -33.3$ (*c* 0.22, $CHCl_3$); MS (FAB): $m/z = 897$ $(M+H)^+$; IR (KBr): ν cm^{-1} 1722 (>C=O), 2858 and 2937 (CH_3 and CH_2 stretching), 3022 (–NH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.33 (m, 10H, Ar–H), 5.94 (d, $J = 3.9$ Hz, 1H, H-1), 4.69 (d, $J = 11.8$ Hz, 1H, –OC$\underline{H}_A$Ph), 4.64 (d, $J = 3.9$ Hz, 1H, H-2), 4.45 (d, $J = 11.8$ Hz, 1H, –OC$\underline{H}_B$Ph), 4.20 and 4.16 (dd, $J = 3.1$ Hz and 8.7 Hz, 1H, H-4), 4.09 (q, $J = 7.1$ Hz, 2H, –OC$\underline{H}_2$CH$_3$), 3.93 (d, $J = 3.1$ Hz, 1H, H-3), 3.41 (m, 1H, H-5), 2.62 (m, 2H, NHC$\underline{H}_2$), 2.42 and 2.34 (dd, $J = 4.8$ Hz and 14.9 Hz, 1H, H-6$_A$), 2.30 and 2.22 (dd, $J = 6.4$ Hz and 14.9 Hz, 1H, H-6$_B$), 1.67 (bs, 1H, -NH), 1.48 and 1.32 [each s, each 3H, 2 × >C(C$\underline{H}_3$)$_2$], 1.24 (m, 13H, CH_2's and –OCH$_2$C$\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 172.23 (>C=O), 137.52, 128.89, 128.45 and 128.27 (Ar–C), 112.04 [>C(CH$_3$)$_2$], 105.23 (C-1), 82.37 (C-2), 82.25 (C-4), 82.18 (C-3), 71.88 (–OCH$_2$Ph), 60.75 (–OCH$_2$CH$_3$), 54.47 (C-5), 47.71 (2 ×–NCH$_2$), 36.69 (C-6), 30.75, 30.03, 29.97, 29.67 and 27.74 (CH_2's), 27.16 and 26.74 [2 × >C (C$\underline{H}_3$)$_2$], 14.59 (–OCH$_2$CH$_3$). Anal. calcd for $C_{50}H_{76}N_2O_{12}$: C, 66.96; H, 8.48; N, 3.12; Found: C, 65.57; H, 8.12; N,3.41.

Antifilarial evaluation

Material and methods. Adult bovine filarial worms *S. cervi* females of average body weight 35 ± 5 mg and length 6.0 ± 1.0 cm were collected from the peritoneal cavity of freshly slaughtered naturally infected water buffaloes *Bubalus bubalis* (Linn.) at a local abbatoir. After being washed with sterile saline, they were allowed to revive for 1 h in 200 mL Hanks Balanced Salt Solution (HBSS) containing 1 μg/mL Gentamycin. Three fully motile female filarial worms were transferred to conical flasks having 25 mL of HBSS supplemented with 5 mM glucose, 100 μg/mL streptomycin sulphate and 300 units/mL pencillin G. The test compound was added at 250 μM concentration. Controls were run in parallel. The flasks were incubated at 37 °C in Dubnoff Metabolic Shaker for desired period with or without the study compound. After desired period of incubation,

the worms were removed from the flask, washed thoroughly with ice cold saline, either a 5.0% homogenate of the worms was prepared in 2.5% sulfosalicylic acid and termed as crude homogenates for glutathione (GSH) estimation[18] or a 10% homogenate of the worms was prepared in isotonic saline (150 mM KCl) for the effect of study compounds on γ-glutamyl cysteine synthetase (γ-GCase),[19] glutathione reductase (GR)[20] and glutathione-*S*-transferase(s) (GSTs)[21] activities, respectively.

Determination of GSH. GSH was determined by reverse-phase HPLC using Spherisorb S 5 μM ODS2 column. 50 μL aliquots of the 10,000 g supernatant were mixed with 50 μL of *O*-phthaldehyde and the reaction was stopped after one min with 100 μL of 0.1 M potassium phosphate $-H_3PO_4$ (pH 7.0). The OPA reagent is composed of 40 mM OPA and 0.4 M sodium tetraborate (pH 9.0) 50 μL of the clear supernatant was injected to the column for GSH analysis. Separation was performed at a flow rate of 1 mL/min with solvent A (0.15 M sodium acetate, pH adjusted to 7.0 with acetic acid/methanol (1/24, v/v) and solvent B (100% methanol). Gradient used was (expressed as percentage of solvent B): 8 min, 0%; 12 min, 10%; 13 min, 25%; 27 min, 90%; 35 min, 90%. Methanol was allowed to come to 0% in 5 min. Solvent A was kept at 100% for equilibration. Effect of title compounds (**3–9** and **11–18**) on intracellular GSH levels of filarial worms is depicted in Table 2.

Determination of γ-GCase. γ-GCase activity was estimated in crude homogenates by a coupled enzyme procedure in which the rate of ADP formation in the presence of pyruvate kinase, LDH, PEP and NADH is obtained from the decrease in absorbance of NADH at 340 nm. L-α-aminobutyrate is used in place of L-cysteine to avoid complications associated with oxidation of L-cysteine, which is not a substrate.

The standard assay system (final volume 1.0 mL) contained Tris–HCl buffer (100 mM, pH 8.4), 150 mM KCl, 5 mM ATP, 2 mM PEP, 10 mM L-glutamate, 10 mM L-α-aminobutyrate, 20 mM $MgCl_2$, 2 mM Na_2EDTA, 0.2 mM NADH, 1.6 μg/μL Pyruvate Kinase and 1.6 μg/μL lactate dehydrogenase. Reaction was initiated by the addition of NADH. The absorbance at 340 nm was monitored for 5 min at 30-s intervals.

Determination of glutathione reductase. The standard reaction mixture for GR activity determination consisted of 100 mM potassium phosphate buffer, pH 7.0; 1 mM EDTA; 10 mM GSSG; 0.2 mM NADPH and enzyme protein. Reaction was initiated by the addition of NADPH. The absorbance at 340 nm was monitored for 5 min at 30-s intervals.

Determination of glutathione-*S*-transferase. The standard reaction for GST activity determination consisted of a mixture of 100 mM phosphate, pH 6.5, 1.0 mM 1-chloro-2, 4-dinitro benzene (CDNB) in 20 μL ethanol, 1.0 mM GSH. Enzyme protein was omitted from the reference cuvette. The formation of GSH conjugated product was measured at 340 nm in Schimadzu double beam spectrophotometer maintained at 25 °C.

Enzyme-inhibition studies. Effect of compounds and standard inhibitors (BSO, cystamine, menadione carmustine) on intracellular glutathione content by adding them in the in vitro maintenance medium at 10–250 μM concentration. However, effect of the above on γ-GCase, GR and GST activity of *S. cervi* females was studied by adding them directly in the assay system (200, 200 and 100 μM, respectively) 10 min prior to addition of the substrate. The percentage inhibition/stimulation of the enzyme activity by compounds was calculated by comparing with control tube. Protein in the samples was determined according to earlier method.[22] In vitro activity of test compounds was evaluated against lymphatic filarial worms *B. malayi* using motility and MTT reduction potential assays as parameters according to the method described by Murthy and Chatterjee.[23] The test compounds were dissolved in DMSO but the final concentration was always kept below 0.1%.

Determination of in vitro antifilarial effect. Micro- and macrofilaricidal activities were evaluated according to methods as reported earlier.[24,25] Microfilarial counts in 10 μL blood were made just before the start of treatment and thereafter at weekly intervals till the 13th week in the case of M. coucha and 9th week in the case of *M. unguiculatus*. Alteration on mf count in relation to pretreatment level was calculated to ascertain the effect on microfilariaemia and fall in microfilaricidal efficacy of the test compounds. On the 13th or 9th week the treated and control animals were sacrificed and adult worms recovered were examined under microscope for motility and mortality. The percent mortality was calculated by comparing the number of live worms recovered from experimental animals with that recovered from matched untreated infected controls. All the surviving female worms recovered from each animal were tested individually on a glass slide and observed under microscope for condition of the intrauterine contents. Female worms with empty uteri or uteri containing the dead or degenerated developmental stages, including mf, were considered as sterilized worms. The percent sterilization of female worms was calculated from the number of worms sterilized out of total live female worms recovered. The concentration of compounds used in the incubation was 100 μM. Motility assay was performed with the adult worm of *B. malayi* recovered from peritoneal cavity of 4–5 months old infected jird (Meriones unguiculatus). The worms were washed in RPMI 1640 medium (pH 7.2) containing streptomycin (100 μg/mL) and penicillin (100 Units/mL). One male and one female worm were kept in 2.0 mL medium (fortified with 10% fetal calf worm) per well with or without test compounds in sterilized 24 well plate (Laxbro, India) were incubated for 24 h at 37 °C.

The control received well an equal amount of vehicle only. Two replicates were set-up for each compound and vehicle. The worms were examined under dissecting microscope after 1, 2, 3, 4 and 24 h of incubation and the condition of the worm was scored as active (+3), sluggish (+2), paralysed (+1) and dead (0). The effect of compounds on motility of adult worms is depicted in Table 3.

The MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was performed as described earlier.[23] The worms incubated in medium containing compound and vehicle as described above for motility assay were used for MTT reduction assay also. The parasites were blot dried over filter paper and kept in 0.1 mL MTT solution (0.5% MTT in PBS) for 1 h at 37 °C followed by solubilization in DMSO (0.1 mL) for 1 h at 37 °C and volume of the solution was made up to 1 mL with DMSO. The absorbance of formazan was measured at 510 nm. The mean absorbance values attained in the replicates were compared with controls. Inhibition in the MTT reduction potential of the parasites was calculated and expressed as percent of control values. Compound producing a motility score of '0' and more than 25% inhibition in MTT reduction is considered as a positive antifilarial compound. The effect of MTT assay is depicted in Table 3.

In vivo studies. For in vivo studies three compounds were selected based on their effect either on enzymes or on the motility and MTT reduction assays. In vivo micro, macrofilaricidal activity and sterilization effect was determined in two different host systems namely jird (M. unguiculatus, WHO recommended model) and with *M. coucha* harbouring 5–6 months old *B. malayi* infections for this study. Transplantation induced establishment of infection in jirds was done as reported earlier.[26] Briefly, 8–10 weeks old male jirds were transplanted intraperitoneally with the adult worms isolated from peritoneal cavity of 4–5 month old experimentally infected jirds.[27] Three animals were used at each dose level in each case and they were kept in control cages. Each animal received 10 female and five male worms. On day 2 or 3 of adult worm transplantation, the peritoneal fluid was aspirated and checked for the presence of mf. The treatment was started on day 7 or 8 post transplantation. L_3 induced *B. malayi* infection on the other hand was produced by inoculating 6–8 week male *M. coucha* with L_3 obtained from experimentally infected laboratory bred black eyed susceptible strain of *Aedes aegypti* mosquitoes which were fed 9/10 days before on microfilaemic *M. coucha*. Each animal received 100 L^3 through subcutaneous route.[28] The intraperitoneal administration of test substance and vehicle was done twice in a day for 10 consecutive days. Treatment of model infection (*B. malayi* in *M. coucha*) with the compounds at 50 mg/kg intraperitoneal doses for 5 consecutive days.

Acknowledgements

The authors are thankful to Director CDRI for his kind consent to undertake this work and to Volkswagen foundation and ICMR New Delhi for financial assistance and to CDRI RSIC staff for spectral data and microanalysis.

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