PURINE NUCLEOSIDE ANALOGUES. 10.*
A NEW SYNTHETIC ROUTE TO 8-SUBSTITUTED-7(9)-ALKOXYALKYLGUANINES**

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Abstract: The N-alkoxyalkylation of 8-bromo-N^2-acetylguanine 1 with α-halogen and α-acetoxy ethers has been investigated. Treatment of 1 with 2-haloethyl chloromethyl ethers in the presence of potassium carbonate afforded 8-bromo-7- and 8-bromo-9-alkoxyalkyl-N^2-acetylguanines in high overall yields. Without added base the replacement of the bromine atom for chlorine at the 8 position of the heterocycle occurred providing 8-chloro-7- and 8-chloro-9-alkoxyalkylated products. The acid catalyzed reaction of 1 with 2-acetoxyethyl acetoxymethyl ether afforded 8-bromo-7- and 8-bromo-9-(2-acetoxyethoxymethyl)-N^2-acetylguanines.

Introduction

The discovery of the potent and selective antiherpetic drug acyclovir, i.e. 9-(2-hydroxyethoxymethyl)guanine, has stimulated substantial efforts in acyclic nucleoside analogues in general and in the N-alkoxyalkylguanines in particular. As a result, many acyclovir congeners have been synthesized with various modifications both in the acyclic side chain and in the heterocycle. These investigations have given rise to several new biologically active compounds. Some 8-substituted (i.e. 8-bromo-, 8-iodo-, 8-amino-, 8-methyl-) derivatives of acyclovir have proven to be active antiherpetic agents (3) as well as potent purine nucleoside phosphorylase (PNP, EC 2.4.2.1) inhibitors (4). Up to now the major attention has been paid to 9-alkoxyalkylguanines. The corresponding N_7-regioisomers, often formed alongside with N_9-substituted derivatives in the chemical synthesis, were regarded mostly as undesirable impurities. Only a few attempts of their systematic investigation have been undertaken so far (5). We are unaware of any published reports on the synthesis and biological activity of 8-substituted-7-alkoxyalkylguanines.

The initial objectives of the present work were to develop a preparative method for the synthesis of 8-substituted-7-alkoxyalkylguanines to make them available for the systematic investigation of their biological properties.

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* For the previous communication in this series see ref. (1).

** For the preliminary communication of this material see ref. (2).

Treatment of substrate **1** with α-chloroether **2a** in dimethylformamide containing an equivalent of potassium carbonate resulted in a quantitative conversion of **1** into a mixture of 8-substituted- N-alkoxyalkylated guanines. Chromatographic resolution of this crude reaction mixture afforded two principal products. The first of them turned out to be 8-bromo-7-(2-chloroethoxymethyl)-N^2-acetylguanine **3c** isolated in 38% yield. The structure of compound **3c** was confirmed by ^{1}H NMR spectrum (Table) and the elemental analysis data. The chromatographically less mobile product consisted of two main N-alkoxyalkylated derivatives (in a ratio of 2:1) that slightly differed in their retention time in HPLC as well as in ^{1}H NMR spectra. The major component of the mixture was isolated in a pure state that allowed to establish its structure as 8-bromo-9-(2-chloroethoxymethyl)-N^2-acetylguanine **4c**. The structure of the minor product was determined in an indirect way, i. e. by a detailed comparison of ^{1}H NMR and mass spectra of the mixture as well as the retention times of its components in HPLC with the same physico-chemical characteristics of the previously isolated pure derivatives **3a-c**, **4a**. Based on this comparison the structure of 8-chloro-9-(2-chloroethoxymethyl)-N^2-acetylguanine **4a** was proposed for this compound. The alkoxyalkylation of **1** with α-chloroether **2b** in the presence of potassium carbonate followed the same course producing 8-bromo-7-(2-bromoethoxymethyl)-N^2-acetylguanine **3d** and 8-bromo-9-(2-bromoethoxymethyl)-N^2-acetylguanine **4d** mostly contaminated with the corresponding 8-chloro counterpart **4b**.

The formation of 8-chloro-9-alkoxyalkylguanines **4a,b** in the N-alkoxyalkylation of **1** in the presence of potassium carbonate is difficult to explain. It is also not quite clear why the analogue 8-chloro-7-alkoxyalkyl substituted compounds **3a,b** were not formed alongside with the corresponding 8-bromo-7-alkoxyalkylguanines **3c,d**. Moreover, it should be pointed out that a scrupulous analysis of the **4b,d** mixture by HPLC revealed also the presence of small quantities of N-alkoxyalkylated products **4a,c**, i.e. derivatives with chlorine in the acyclic moiety. At the same time the alkylating agent **2b** did not contain any traceable amount of ether **2a** according to glc data. These experimental facts may suggest an interaction between halogens in the acyclic moiety and the 8 position of the heterocycle (perhaps formation of some cyclic intermediate products) characteristic just for 8,9-disubstituted guanines **4a-d**. This assumption was partly supported by our experiments with octyl chloromethyl ether **5a** and octadecyl chloromethyl ether **5b**. When the guanine **1** was treated with ethers **5a,b** in the presence of anhydrous potassium carbonate the corresponding 8-bromo-7- and 8-bromo- 9-(2-octyloxymethyl)-N^2-acetylguanines **6a,7a** and 8-bromo-7- and 8-bromo- 9-(2-octadecyloxymethyl) -N^2-acetylguanines **6b,7b** were isolated. No 8-chloro substituted analogues were detected in this case.

When compound **1** was treated with 2-acetoxyethyl acetoxymethyl ether **8** in hot

Results and Discussion

Our attempt to prepare 7,8-disubstituted guanines by halogenation at the 8 position of previously obtained 7-alkoxyalkyl derivatives , i.e. by the route commonly used for the synthesis of 8,9-disubstituted guanines (3), failed. Therefore, to obtain the target 7,8-disubstituted derivatives we decided to alkylate 8-bromo-N^2-acetylguanine. It has been demonstrated that the aralkylation of this substrate with benzyl bromide proceeds with a better yield than that of N^2-acetylguanine and affords 8-bromo-7-benzylguanine as one of the major reaction products (1). It encouraged us to extend this method to the preparation of 8-bromo-7-alkoxyalkylguanines.

When 8-bromo-N^2-acetylguanine **1** (1) reacted with 2-chloroethyl chloromethyl ether **2a** in dimethylformamide at room temperature, two main alkoxyalkylation products were isolated in about 20% and 40% yield respectively. Although their ^{1}H NMR spectra were consistent with the structures of 8-bromo-7(9)-(2-chloroethoxymethyl)-N^2-acetylguanines **3c**, **4c**, the elemental analysis data did not support this assignment. Mass spectra (FAB and EI normalized according to ^{35}Cl and ^{79}Br) were used to solve this discrepancy. The intensities of isotope peaks of the molecular ion of the chromatographically less mobile product m/z 319 (100), m/z 321 (66.71), m/z 323 (13) corresponded to the presence of two chlorine atoms in the molecule. Based on these data the structure of 8-chloro-9-(2-chloroethoxymethyl)-N^2-acetylguanine **4a** was assigned to this compound and that of 8-chloro-7-(2-chloroethoxymethyl)-N^2-acetylguanine **3a** to the other . The site of alkoxyalkylation (N_7- or N_9-) was confirmed by the chemical shift of NCH_2-group two- protons singlet observed in their ^{1}H NMR spectra (Table), namely, a downfield shift of the signal for 7-isomer **3a** (5.74 ppm) in comparison with the same signal for 9-isomer **4a** (5.49 ppm). A similarly conducted alkoxyalkylation of **1** with 2-bromoethyl chloromethyl ether **2b** yielded the corresponding 8-chloro-7-(2-bromoethoxymethyl)- and 8-chloro-9-(2-bromoethoxymethyl)-N^2-acetylguanines **3b**, **4b**. ^{1}H NMR spectral data for **3b**, **4b** are presented in the table. The intensities of isotope peaks of the molecular ion of **4b** m/z 363 (27.52), m/z 365 (36.62), m/z 367 (10.49) corresponded to the presence of one chlorine and one bromine atom in the molecule.

The results indicate that two main processes occur under these reaction conditions: the alkoxyalkylation at the imidazole ring nitrogen atoms and the substitution of bromine at the 8 position of the heterocycle for chlorine. Although the susceptibility of bromine at the 8 position of guanine to such substitution was observed in the reaction of 8-bromo-9-(2-hydroxyethoxymethyl)guanine with thionyl chloride (6) this replacement was not expected to occur under the mild conditions of alkoxyalkylation. The process could be explained assuming that it was caused by hydrogen chloride formed both in the reaction with thionyl chloride and in the alkylation with α-chloroethers.

dimethylformamide (100-120°C) with added catalytical amount of p-toluenesulphonic acid 8-bromo-7- and 8-bromo-9-(2-acetoxyethoxymethyl)-N^2-acetylguanines **9**,**11** were isolated in 40% and 32% yield respectively. In some cases a very small amount of dialkoxyalkylated derivative **10** was obtained. Its proposed structure was assigned by ^{1}H NMR spectrum (Table). The features of the spectrum were very close to those observed in ^{1}H NMR spectrum of 8-oxo-7,8-dihydro-7,9-dibenzyl-N^2-acetylguanine obtained in the aralkylation of **1** with benzyl bromide (1). Therefore, the structure of 8-oxo-7,8-dihydro-7,9-di-(2-acetoxyethoxymethyl)-N^2-acetylguanine was assigned to compound **10** as the most probable one. The overall yield of this product was too small to investigate it in detailes.

The 8-bromo-7-alkoxyalkylated guanine **9** was successfully used as an intermediate for the synthesis of some other 7,8-disubstituted guanine derivatives . N-Deacetylation of **9** with aqueous methylamine afforded 8-bromo-7-(2-hydroxyethoxymethyl)guanine **12**. The reaction of the same substrate with sodium thiosulphate in the presence of aluminium chloride (7) yielded 8-mercapto-7-(2-acetoxyethoxymethyl)-N^2-acetylguanine **13** which was deacetylated to give 8-mercapto-7-(2-hydroxyethoxymethyl)guanine **14**.

In summary, it is possible to conclude that the direct alkoxyalkylation of 8-bromo-N^2-acetylguanine with α-halogen or α-acetoxy ethers provides a new synthetic route to 8-substituted-7-alkoxyalkyl guanines. 8-Substituted-9-alkoxyalkylguanines can also be prepared by this method.

Experimental

All reagents were of commercial grade. Reagent grade solvents were used without further purification. The solvent mixtures are in volumes. Melting points were determined on Boetius hot stage microscope and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 400 (400 MHz) instrument in DMSO-d_6 with tetramethylsilane as an internal standard. Mass spectra were run on a V.G. Micromass ZAB-HFqQ Varian mass spectrometer, coupled to a V.G. 11/250 data system. Column chromatography was performed on silica gel L 100/400 (Czech Republic). The results of the elemental analysis of all the compounds obtained are within ±0.4 % of the theoretical values.

8-Chloro-7-(2-chloroethoxymethyl)-, 8-chloro-7-(2-bromoethoxymethyl)-, 8-chloro-9-(2-chloroethoxymethyl)-, 8-chloro-9-(2-bromoethoxymethyl)-N^2-acetylguanine 3a,b; 4a,b

A solution of compound **1** (1.36 g; 5 mmol) and α-chloro ether **2a** (0.81 g; 6.25 mmol) in dimethylformamide (50 ml) was stirred at room temperature for 48 h. The reaction mixture was adjusted to pH 8 with conc. aqueous ammonia and evaporated to dryness *in vacuo*. The residue was suspended in chloroform (100 ml). The insoluable material was filtered off, the filtrate was concentrated *in vacuo* and resolved by chromatography on silica gel with chloroform:ethanol, 40:1

to obtain 0.30 g (19%) of 3a, m.p. 183-186°C (ethanol) and 0.69 g (43%) of 4a, m.p. 225°C (ethanol).

Using the procedure described for the synthesis of 3a, 4a products 3b, 4b were obtained from substrate 1 (1.36 g; 5 mmol) and α-chloro ether 2b (1.08 g; 6.25 mmol). Yield - 0.42 g (23%) of 3b, m.p. 175-177 °C (ethanol) and 0.56 g (31%) of 4b, m p. 211°C (ethanol).

8-Bromo-7-(2-chloroethoxymethyl)-, 8-bromo-7-(2-bromoethoxymethyl)-, 8-bromo-9-(2-chloroethoxymethyl)-, 8-bromo-9-(2-bromoethoxymethyl)-N²-acetylguanine 3c,d; 4c,d

Products 3c,d and 4c,d were synthesized from compound 1 (1.36 g; 5 mmol), appropriate α-halogen ether 2a or 2b (6.25 mmol) and K_2CO_3 (0.86 g; 6.25 mmol) using the procedure described for the preparation of 3a,b. Yields - 0.70 g (38%) of 3c, m.p 184-188° C (ethanol); 0.54 g (30%) of 4c, m.p. 202-204°C (ethanl); 0.98 g (48%) of 3d, m.p. 167-168°C (ethanol); 0.41 g (20%) of 4d, m.p. 194-196°C (ethanol).

8-Bromo-7-(2-octyloxymethyl)-, 8-bromo-9-(2-octyloxymethyl)-N²-acetylguanine 6a, 7a

Products 6a, 7a were synthesized from compound 1 (1.14 g; 4 mmol), α-chloro ether 5a (0.94 g; 5 mmol) and K_2CO_3 (0.72 g; 5 mmol) using the procedure described for the preparation of 3a, 4a. Recrystallization from ethanol afforded 0.55 g (33%) of 6a, m.p. 179°C and 0.23 g (14%) of 7a, m.p. 215°C.

8-Bromo-7-(2-octadecyloxymethyl)-, 8-bromo-9-(2-octadecyloxymethyl)-N²-acetyl-guanine 6b; 7b

A mixture of compound 1 (0.9 g; 3.3 mmol), α-chloroether 5b (1.32 g; 4.1 mmol) and K_2CO_3 (0.57 g; 4.1 mmol) in dimethylformamide (30 ml) was stirred at 55-60°C for 15 h. The precipitate was filtered off to yield 1.0 g of a crystalline substance containing regioisomers 6b and 7b in a ratio of 1:1. The separation of these compounds was carried out by chromatography on silica gel with chloroform to obtain 0.38g (21%) of 6b and 0.48 g (26%) of 7b.

An additional quantity of 6b, 7b was obtained from dimethylformamide filtrate. Its evaporation to dryness *in vacuo* and the resolution of the residue by chromatography on silica gel with chloroform afforded 0.25 g (14%) of 6b and 0.14 g (8%) of 7b. The overall yield - 0.63 g (35%) of 6b, m.p. 159-160°C (ethanol) and 0.62 g (34%) of 7b, m.p. 205-206°C (ethanol).

8-Bromo-7-(2-acetoxyethoxymethyl)-, 8-oxo-7,8-dihydro-7,9-di-(2-acetoxyethoxy-methyl)-, 8-bromo-9-(2-acetoxyethoxymethyl)-N²-acetylguanine 9,10, 11

A solution of compound 1 (2.0 g; 7.4 mmol), α-acetoxy ether 8 (1.94 g; 11 mmol) and p-toluenesulfonic acid monohydrate (0.04g; 0.2 mmol) in dimethylformamide (50 ml) was stirred at 120°C for 6 h. The reaction mixture was cooled to room temperature and evaporated to dryness *in vacuo*. The residue was suspended in chloroform (100 ml), the insoluable material was filtered off,

the filtrate was concentrated *in vacuo* and resolved by chromatography on silica gel with chloroform : ethanol, 40:1 to give 1.15 g (40%) of **9**, m.p. 184-185°C (ethanol) , 0.08 g (2.5%) of **10** and 0.92 g (32%) of **11**, m.p. 212-214°C (ethanol).

8-Bromo-7-(2-hydroxyethoxymethyl)guanine 12

A solution of compound **9** (0.1 g; 0.26 mmol) in 25% methylamine water solution (10 ml) was stirred at room temperature for 4 h. The reaction mixture was evaporated to dryness *in vacuo*. The residue was recrystallized from water affording 0.07 g (90%) of product **12**, m.p. >280°C (decomp.).

8-Mercapto-7-(2-acetoxyethoxymethyl)-N^2-acetylguanine 13

A suspension of compound **9** (0.5 g; 1.3 mmol), $Na_2S_2O_3x\ 5H_2O$ (1.2 g; 4.84 mmol) and $AlCl_3x\ 6H_2O$ (0.07g; 0.3 mmol) in water (30 ml) was refluxed for 4h and cooled to room temperature. The precipitate was separated by filtration and recrystallized from water to give 0.28 g (69%) of product **13**, m.p. >260°C (decomp.).

8-Mercapto-7-(2-hydroxyethoxymethyl)guanine 14

A solution of compound **13** (0.15 g; 0.4 mmol) in 25% methylamine water solution (10 ml) was refluxed for 0.5 h . The reaction mixture was cooled to room temperature and evaporated to dryness *in vacuo*. The residue was recrystallized from water to give 0.93 g (89%) of derivative **14**, m.p. >280°C (decomp.).

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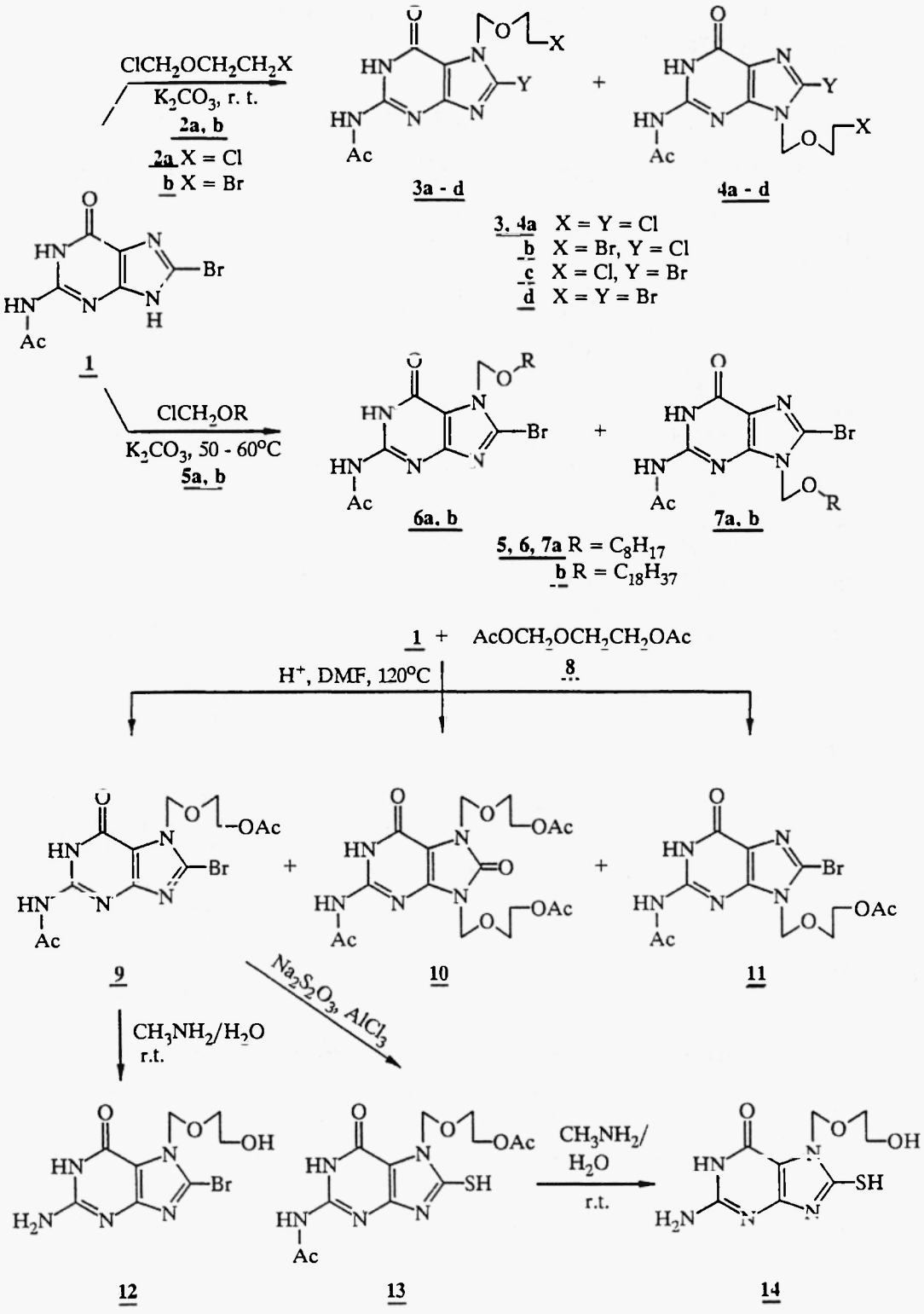
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K2CO3, r. t.
2a, b
2a X = Cl
b X = Br
3a - d
4a - d
3, 4a X = Y = Cl
b X = Br, Y = Cl
c X = Cl, Y = Br
d X = Y = Br
1
ClCH2OR
K2CO3, 50 - 60°C
5a, b
6a, b
7a, b
5, 6, 7a R = C8H17
b R = C18H37
1 + AcOCH2OCH2CH2OAc
8
H+, DMF, 120°C
9
10
11
Na2S2O3, AlCl3
CH3NH2/H2O
r.t.
12
13
14

T A B L E ^{1}H NMR Chemical Shifts (δ,ppm) of N-Alkoxyalkylated Derivatives of 8-Substituted Guanines **3**,**4a-d**; **6**,**7a,b**; **9-15**

Compound	NH (s,1H)	NCH_2 (s,2H)	CH_2CH_2 2x (m,2H)	$COCH_3$ (s,3H)	Other protons
3a	12.24 11.68	5.74	3.78 (m,4H)	2.19	-
3b	12.23 11.67	5.72	3.88 3.57	2.14	-
3c	12.21 11.67	5.71	3.77 (m,4H)	2.17	-
3d	12.18 11.63	5.70	3.89 3.56	2.16	-
4a	12.01 (br.s,2H)	5.49	3.82 3.73	2.20	-
4b	12.16 11.78	5.46	3.82 3.58	2.17	-
4c	12.01 (br.s,2H)	5.46	3.82 3.74	2.20	-
4d	12.06 11.73	5.43	3.84 3.74	2.18	-
6a	11.15	5.78	-	2.27	3.59(2H,t,OCH_2); 1.49-1.24 (16H, m, CH_2); 0.87(3H, m, CH_3)
6b	12.07 11.64	5.76	-	2.32	3.53(2H,m,OCH_2); 1.48-1.24 (36H, m CH_2); 0.86 (3H, m, CH_3)
7a	10.87	5.41	-	2.29	3.47(2H,t,OCH_2); 1.49-1.22 (16H, m CH_2) 0.87(3H,m,CH_3)
7b	12.07 11.74	5.39	-	2.16	3.49(2H,m,OCH_2); 1.21(36H,m,CH_2); 0.86(3H,m ,CH_3)
9	11.98 11.74	5.38	4.03 3.70	2.16 1.91	-
10	11.91	5.28 5.12	4.03 3.69	2.13 1.93	-
11	12.13 11.61	5.63	4.02 3.72	2.13 1.93	-
12	10.91	5.60	3.52 (m,4H)	-	6.31(2H,s,NH_2); 4.62(1H,m,OH)
13	13.47;12.14 11.82	5.63	4.06 3.83	2.17 1.97	-
14	10.88	5.56	3.54 (m,4H)	-	6.57(2H,s,NH_2); 4.54(1H,t,OH)