

"BABES-BOLYAI" UNIVERSITY CLUJ-NAPOCA FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING ORGANIC CHEMISTRY DEPARTMENT

CRINA-ANCA CISMAŞ

Synthesis and Structural Analysis of Some New Derivatives with Saturated Six-membered Heterocycles and the Synthesis of Some New Amphiphilic Purine Derivatives

PhD THESIS Abstract

Scientific Adviser, **Prof. Univ. Dr. ION GROSU**

JURY

PRESIDENT

Prof. Univ. Dr. LUMINIȚA SILAGHI-DUMITRESCU – Dean of the Faculty of Chemistry and Chemical Engineering, "Babes-Bolyai" University Cluj-Napoca

REVIEWERS

Prof. Univ. Dr. JUERGEN LIEBSCHER – Humboldt Universitaet zu Berlin (Germany)

Prof. Univ. Dr. SORIN ROȘCA – "Politehnica" University Bucuresti (Romania) Prof. Univ. Dr. SORIN MAGER - "Babes-Bolyai" University Cluj-Napoca (Romania)

Defence: 19th October, 2004

CONTENTS

INTRODUCTION

1

PART A				
SIX-MEMBE	RED I		ES:	
CARBOCYC	LES	AND HETERO	CYCLES CONTAINING OXYGEN	2
1.	Gene	eralities conce	erning the spirane compounds	2
2.	Class	sification of si	x-membered rings spiranes	2
3.	Stere	echemical as	pects	
	of siz	x-membered r	ings spirane compounds	4
	3.1	Configuratio	onal isomers	
	of sp	oiranes with si	ix-membered rings	4
	3.2	Conformatio	onal behavior	
	of sp	oiranes with si	ix-membered rings	12
		3.2.1	Flexible spiranes	12
		3.2.2	Semiflexible spiranes	13
		3.2.3	Anancomeric spiranes	17
4.	Six-n	nembered rinç	gs spiranes	
	exhib	biting only car	bocycles	19
5.	Oxyg	gen containing	g six-membered rings spiranes	22
	5.1	One oxygen	atom in the spirane skeleton	22
		5.1.1	Monospiranes	22
	5.2	Two oxygen	atoms in the spirane skeleton	26
		5.2.1	Monospiranes	26
	5.3	Three oxyge	en atoms in the spirane skeleton	29
		5.3.1	Monospiranes	29
		5.3.2	Dispiranes	29
	5.4	Four oxyger	n atoms in the spirane skeleton	33
		5.4.1	Monospiranes	33
		5.4.2	Dispiranes	36
		5.4.3	Tri-, tetra-, penta- and hexa-spiranes	39
6.	Cond	lusions		41
References				42

References

PART B

SYNTHESIS	AND	STEREOCHEMISTRY	
OF SOME N	EW B	ROMINATED 1,3-DIOXANE DERIVATIVES	45
1.	Brom	nination of Cyclic Acetals	45
2.	Syntl	hesis and Stereochemistry	
	of the	e 1,3-Dioxane Intermediates	49
3.	Syntl	hesis and Structural Analysis	
	of Ne	w Brominated Derivatives	57
	3.1	Monobrominated compounds 16-20	57
	3.2	Dibrominated compounds 21-26	67
4.	Expe	rimental Part	75
References			87

PART C

SYNTHESIS	AND CONFORMATIONAL ANALYSIS	
OF SOME B	S(1,3-OXATHIANE-2-YL) DERIVATIVES	90
1.	General Aspects Concerning the Stereochemistry of	
	1,3-Oxathiane Derivatives	90
2.	Synthesis of New Bis(1,3-oxathiane-2-yl) Derivatives	94
3.	Structural Aspects in Solution	96
	3.1 Conformational Aspects	96
	3.2 Configurational Aspects	101
4.	Structural Aspects in Solid State	107
5.	Synthesis of Oxides of Bis(1,3-oxathiane-2-yl) Derivatives	109
6.	Experimental Part	112
References		121

PART D

SYNTHESIS	OF NEW AM	PHIPHILIC PURINI	E NUCLEOSIDES
-----------	-----------	------------------	----------------------

- 1.General Introduction. Scopes and Strategies123
- 2. Synthesis of Some New C-8 Purine Derivatives 127
- 3. Attempts on Synthesis of Derivatives

Bearing more Lipophilic Substituents	131
4. Attempts on the Synthesis	
of Protected 8-Modified-2'-deoxyadenosine Derivatives	
 – for their Use in Automated DNA Synthesis 	137
5. Experimental Part	142
6. Acknowledgments	158
References	159

CONCLUSIONS LIST OF PRODUCTS

162

INTRODUCTION

The research work presented in this Ph.D. Thesis is structured in four main parts. The first chapter (Part A) is dedicated to the exhaustive literature investigation of the sixmembered rings spiranes – carbocycles and oxygen containing spiro-compounds. The most important data concerning the synthesis and the stereochemistry of these derivatives are reviewed.

The original work was developed in the field of the synthesis and the stereochemistry of new 1,3-dioxanes derivatives (Part B), synthesis and conformational analysis of some bis(1,3-oxathiane-2-yl) derivatives (Part C) and the synthesis of some new amphiphilic purine nucleosides (Part D). The research for the first parts was developed at "Babes'Bolyai" University, Cluj-Napoca, while the investigations for the last one were conducted in the research group of Prof. J. Liebscher at Humboldt Universitaet zu Berlin during a 12 months research stage financed by a DAAD fellowship.



The research concerning the saturated six-membered rings heterocycles was focused on the study of the stereochemistry of new chiral mono- (I) and dibrominated (II) 1,3-dioxanes and the investigation of the stereochemistry and of the ring-chain tautomerism *via like-unlike* equilibrium of some new derivatives III.

The aims of the research on purine nucleosides were dedicated to the synthesis of new amphiphilic derivatives IV and V and to the study of their applications as highly functional biological surfaces.

4

PART B

SYNTHESIS AND STEREOCHEMISTRY OF SOME NEW BROMINATED 1,3-DIOXANE DERIVATIVES

2. Synthesis and Stereochemistry of the 1,3-Dioxane Intermediates

Derivatives **4-15** (among them, six (**4-9**) are new compounds¹ and four of them have already been described in the literature **10-13**²) were obtained by the condensation reaction of the appropriate ketones or aldehydes with 1,3-propanediols, in acidic conditions (PTSA) in benzene; the equilibrium was shifted towards the product by azeotropic removal of the formed water.³

R ₁	HO R ₃	H ⁺ R₁	
R ₂ +	HO—R ₄	-H ₂ O R ₂	O R ₄
$R_1 = C_6 H_5 C H_2$	R ₂ =H	$R_3=R_4=CH_3$	4
$R_1 = C_6 H_5 C H_2$	R ₂ =H	$R_3=R_4=COOC_2H_5$	5
$R_1 = C_6 H_5 C H_2$	$R_2 = C_6 H_5$	$R_3=R_4=CH_3$	6
$R_1 = C_6 H_5 C H_2$	$R_2 = C_6 H_5$	$R_3=R_4=COOC_2H_5$	7
R_1 = CH_3CH_2	$R_2 = C_6 H_5$	$R_3=R_4=CH_3$	8
$R_1 = CH_3CH_2$	$R_2 = C_6 H_5$	$R_3=R_4=COOC_2H_5$	9
$R_1 = R_2 = CH_3CH_2$	R₃=H	$R_4 = CH_3$	10
$R_1 = R_2 = CH_3CH_2CH_2$	R₃=H	$R_4 = CH_3$	11
$R_1 = R_2 = C_6 H_5 C H_2$	R ₃ =H	$R_4 = CH_3$	12
R ₁ + R ₂ = (CH ₂) ₄	R ₃ =H	$R_4 = CH_3$	13
R ₁ + R ₂ = (CH ₂) ₅	R ₃ =H	$R_4 = CH_3$	14
R ₁ + R ₂ = (CH ₂) ₆	R₃=H	$R_4 = CH_3$	15

Scheme 1

Studies on the stereochemistry of 2-alkyl-1,3-dioxanes and of 2-alkyl-2-aryl-1,3dioxane derivatives^{4,5,6,7,8} showed a high conformational free enthalpy for the methyl group – or substituted methyl groups – (ΔG_{Me} =3.8-3.9 kcal/mol).^{6,9,10,11} Compared to a methyl groups, the phenyl substituent displays lower value of conformational free enthalpy (ΔG_{Ph} =3.12 kcal/mol). In 2-methyl (or substituted methyl)-2-phenyl-1,3-dioxanes, the phenyl group shows a higher preference for the axial orientation (ΔG°_{Me-Ph} = 2.42 kcal/mol)¹¹ than calculated by simple addition of the conformational free enthalpies measured for the two groups in monosubstituted compounds ($\Delta G^{\circ}_{Me} - \Delta G^{\circ}_{Ph} = 0.8$ kcal/mol).⁶

These data show that the conformational equilibrium for the investigated type of compounds is shifted toward the conformer displaying the $-CH_2-X$ substituent (X= -CH₃, - C₆H₅) in equatorial position and all the derivatives exhibit anancomeric structures (Scheme 2).



Scheme 2

The rigid structure of the compounds is illustrated in ¹H-NMR spectra by the appearance of different sets of signals for axial and equatorial protons at positions 4 and 6 of the heterocycle, as well as for protons of the axial and equatorial methyl or ethyloxycarbonyl groups located in the aliphatic moiety of the 1,3-dioxane ring. Usually the equatorial C4, C6 protons are more deshielded than the axial ones, while in the case of the identical groups located at C5, the protons and the carbon atoms of the axial group are the more deshielded ones.^{12,13,14,15,16,17} For the carbon atoms, situated in α to C5, the deshielding is the result of the γ_{anti} effect.^{18,19,20}

Table 1 presents NMR data for compounds **4-9**, namely the chemical shifts for the protons at positions 4 and 6 and for the protons belonging to the groups located at position 5 of the heterocycle along with the differences in chemical shifts between equatorial and axial orientations. It is worth noticing that the differences between the chemical shifts of the protons at positions 4 and 6 are larger for the compounds bearing ester groups than for the compounds with methyl group located at position 5 of the 1,3-dioxane ring. It is also notable the ASIS effect on the $\Delta\delta$ of the protons of the methyl groups as the solvent is changed from chloroform- d_1 to benzene- d_6 (compounds **6** and **8** for example).

Comp.	$\delta H_{4(6)ax}$	$\delta H_{4(6)eq}$	$\Delta \delta_{\text{ax-eq}}$	δ (-CH ₂ -) _{5ax}	δ (-CH ₂ -) _{5eq}	$\Delta \delta_{\text{ax-eq}}$	δ (-CH ₃) _{5ax}	δ (-CH ₃) _{5eq}	$\Delta \delta_{\text{ax-eq}}$
4	3.41	3.62	0.19	-	-	-	1.21	0.71	0.50
5	3.91	4.71	0.80	4.32	4.15	0.17	1.30	1.23	0.07
6	3.35	3.42	0.07	-	-	-	1.07	0.53	0.54
6*	3.23	3.35	0.12	-	-	-	1.06	0.09	0.97
7	3.91	4.48	0.57	4.15	4.04	0.11	1.26	1.15	0.11
8	3.46	3.38	0.12	-	-	-	1.27	0.57	0.70
8*	3.26	3.70	0.44	-	-	-	1.24	0.16	1.08
9	3.93	4.48	0.55	4.35	4.06	0.29	1.34	1.16	0.18

Table 1 ¹H-NMR data for compounds **4-9** (δ values in ppm) in CDCl₃ or C₆D₆ (*)

Positions 4 and 6 are homotopic, in ¹³C-NMR spectra only one signal is displayed for those positions. Table 2 presents ¹³C-NMR data for the carbon atoms of the substituents located at position 5 (also with the corresponding $\Delta \delta_{5ax-5eq}$ values) and for the homotopic carbon atoms at positions 4 and 6 of the heterocycle.

Comp.	δC ₄₍₆₎	δ	δ	δ	δ	$\Delta \delta_{ax-eq}$	δ	δ	$\Delta \delta_{ax-eq}$
	()	(-CO-) _{5ax}	(-CO-) _{5eq}	(-CH ₂ -) _{5ax}	(-CH ₂ -) _{5eq}	•	(-CH ₃) _{5ax}	(-CH ₃) _{5eq}	•
4	77.34	-	-	-	-	-	23.07	21.91	1.16
5	69.34	168.0	166.9	62.03	62.03	0.00	14.09	13.98	0.11
6	71.63	-	-	-	-	-	22.77	21.90	0.87
7	63.64	167.8	166.9	61.88	61.83	0.05	14.08	13.89	0.19
8	71.61	-	-	-	-	-	22.71	22.33	0.38
9	63.64	167.0	167.0	61.98	61.98	0.00	14.21	13.90	0.31

Table 2 $^{\rm 13}\text{C-NMR}$ data for compounds 4-9 (δ values in ppm) in CDCl_3

The stronger deshielding of the equatorial protons in compounds **5**, **7** and **9** is due to the influences through space of the oxygen atoms of the axial ester group (Chart 1) that create a deshielding area at the level of the equatorial protons of the heterocycle.



Chart 1 Ethyl "inside" rotamer of compounds 5, 7 and 9

Compounds **6-9** exhibit axial phenyl group. The rotation of the phenyl substituent around its bond with the heterocycle is hindered by the interactions between the aromatic

substituent and the axial hydrogen atoms at positions 4 and 6 of the 1,3-dioxane ring²¹ and thus the preferred rotamer corresponds to the orthogonal arrangement that minimizes the *syn*-axial interactions.

The limited rotation of the aryl group induces the influence of its magnetic anisotropy on the protons of the heterocycle and on the groups located on it. As a result, the differences between the chemical shifts corresponding to the equatorial and axial protons at positions 4 and 6 for these compounds are somewhat smaller than those for the compounds without phenyl groups in their molecule (compounds 4 and 5) - for comparison there are taken into consideration compounds bearing the same substituents at position 5 of the 1,3-dioxane ring (4 with 6 and 5 with 7). The situation is due, on one hand, to the influence through space of the already mentioned anisotropic magnetic field that locate the axial protons at these positions in the deshielding area while the equatorial ones are in the shielding area of the magnetic field – and, on the other hand, to the deshielding of the axial protons in positions 4 and 6 because of the "steric compression" exerted by the axial aromatic group on these protons. For the methyl groups located at position 5 the situation is reversed (as it may be observed in Table 1), the differences in chemical shifts are higher. Also in the ¹³C-NMR spectra the carbon atoms at positions 4 and 6 are more shielded than the same atoms in the compounds without phenyl group in position 2 of the heterocycle.



Chart 2 The axial orthogonal rotamer of compounds 6-9

For the monosubstituted 5-methyl-1,3-dioxane derivatives **10-15**, the conformational analysis revealed anancomeric structures, despite the small value of the conformational free enthalpy of the methyl group located in the aliphatic part of the heterocycle (*e.g.* $\Delta G^{o}_{Me(position5)}=0.83$ kcal/mol).¹⁰ The conformational equilibria are shifted towards the conformations having the 5-methyl group in equatorial orientation (Scheme 3).



Scheme 3

3. Synthesis and Structural Analysis of New Brominated Derivatives

3.1 Monobrominated compounds 16-20

Bromination of the 1,3-dioxanes previously prepared was performed with bromine in conditions similar to those used by Giusti in the bromination of 1,3-dioxolane compounds.^{22,23} The aim of this part of the thesis was to obtain the monobrominated derivatives. The reaction scheme is presented below.²⁴



As in the case of non-brominated 1,3-dioxanes, the molecules exhibit anancomeric structures. For compound **16** the characteristic conformational equilibrium (Scheme 5) is shifted toward the conformer showing the substituent in position 2 in equatorial orientation, whereas the preferred conformer of compounds **17-20** exhibits the phenyl group in axial orientation (Scheme 6).



Scheme 6

The substituted methyl group [as Me-CH(Br)- or Ph-CH(Br)-] has an A value somewhat higher than an unsubstituted methyl group.²⁵ The methyl substituent at position 2 of the 1,3-dioxane ring has equatorial orientation, thus the aromatic substituent in compounds **17-20** presents axial disposition.

The appearance of the chiral centre in the molecule of compounds **16-20** produces the existence of enantiomers and the chirality introduces the diastereotopicity at the positions 4 and 6 of the heterocycle, as observed in the ¹H and ¹³C-NMR spectra. This behaviour is illustrated by more complex NMR spectra, which exhibit different signals for these positions, as well as different signals for the axial and equatorial protons because of the anancomericity of the heterocycle (Table 3 and Table 4).

Comp.	δH_{4eq}	δH_{6eq}	Δδ ₆₋₄	δH_{4ax}	δH_{6ax}	$\Delta \delta_{6-4}$	δ (-CH ₂ -) _{5ax}	δ (-CH ₂ -) _{5eq}	δ (-CH ₃) _{5ax}	δ (- CH ₃) _{5eq}
16	3.30	3.39	0.09	3.00	3.05	0.05	-	-	1.07	0.22
17	3.3	30	-	3.	30	-	-	-	1.34	0.09
17*	3.50	3.54	0.04	3.4	44	-	-	-	1.36	0.58
18	4.93	4.97	0.04	4.07	4.11	0.04	4.16 and 4.19	3.58	1.09	0.63
18*	4.63	4.67	0.04	3.89	3.92	0.03	4.32	4.06	1.37	1.17
19	3.25	3.26	0.01	3.25	3.33	0.08	-	-	1.28	0.09
20	4.85	4.88	0.03	4.	08	-	4.15	3.59	1.03	0.64

Table 3 ¹H-NMR data for compounds **16-20** (δ values in ppm) in C₆D₆ or CDCl₃ (*)

Comp.	δC4	δC4	$\Delta \delta_{6-4}$	δ	δ	δ	δ	δ	δ
				(-CO-) _{5ax}	(-CO-) _{5eq}	(-CH ₂ -	(-CH ₂ -) _{5eq}	(-CH ₃) _{5ax}	(-
) _{5ax}	. ,		CH ₃) _{5eq}
16	76	.69	-	-	-	-	-	22.61	20.82
17	71	.66	-	-	-	-	-	22.90	21.14
18	65	.19	-	168.15	167.32	62.90	62.39	14.88	14.29
18*	64	.07	-	167.53	163.55	62.23	62.20	14.20	13.80
19	71.93	71.97	0.04	-	-	-	-	23.24	21.48
20	65.20	65.33	0.13	168.31	167.34	62.86	62.47	14.96	14.44

Table 4 ¹³C-NMR data for compounds **16-20** (δ values in ppm) in C₆D₆ or CDCl₃ (*)

A remarkable feature is the recording in the ¹H-NMR spectrum of compound **18** (Figure 1) of different signals (δ = 4.16 and δ ' = 4.19 ppm) for the diastereotopic methylene protons of the axial ester group. The pattern shows for these protons two overlapped doublets of quartets (due to the geminal coupling between the diastereotopic protons and to the coupling of these ones with the protons of the vicinal methyl group). The influence of the chiral carbon atom (at a distance of eight bonds) is transmitted through space. The lack of the same differentiation for the similar protons of the equatorial ester group (also located at eight-bond distance of the chiral carbon atom, but showing an usual quartet) can be considered as a proof for this supposition.

COOCH₂CH₃



Figure 1 NMR spectrum (fragment) of compound 18

This conclusion is suggested by the rotameric behaviour of the axial ester group at position 5 and of the chiral substituent located in the ketal part of the heterocyclic ring. The conformer displaying the axial substituent located at C^5 towards the 1,3-dioxane ring is the most representative (this orientation determines, by means of the influence of the oxygen atoms, the observed deshielding of the protons and carbon atoms of this group).

The three rotamers (Chart 3, I – III) of the chiral group located at position 2 exhibit different populations. They generate a sufficient differentiation of the average magnetic environment for the two methylene protons of the axial ester group to permit the recording of different signals for each of them in the ¹H-NMR spectrum recorded in benzene- d_6 . Because the distance between the chiral centre located at position 2 and the equatorial group at position 5 is too large, the differences between the magnetic environments of the diastereotopic equatorial methylene protons are too small to observe their diastereotopicity.



Chart 3 The three possible rotamers of compound 18 (regarding the C2-Ca bond)

3.2 Dibrominated compounds 21-26

New 1,3-dioxane derivatives bearing two identical chiral substituents at position 2 of the heterocycle were synthesized by the dibromination reaction of the compounds **10-15** (Scheme 7). The reactions were carried out in dichloromethane at low temperature to provide diastereoisomers mixtures of different ratios in average yields.²⁶ The ready crystallization from ethanol of the major diastereoisomers allows their isolation in diastereoisomeric pure form (*meso* or *d*,*l*); compound **21** is an exception being isolated as mixture.



Scheme 7

The mechanism²⁷ of the bromination involves the participation of the hydrobromic acid formed in the reaction as catalyst and the formation of cation I and enol ether II. Further, in reaction with bromine, these cationic intermediates give the monobrominated compound III and then the second bromine equivalent attacks the second α position of the molecule to give IV (Scheme 8).



Scheme 8

Compounds **21-23** exhibit anancomeric, rigid structure, as the conformational equilibria are shifted towards the conformations displaying the methyl group at position 5 of the heterocycle in equatorial orientation (Scheme 9).

When dibromination takes place, two new chiral centres are formed and therefore two configurational diastereoisomers are possible: a *meso* isomer (*RS* or *SR* configurations for the chiral carbon atoms) and a racemic *d,I* isomer (*RR* or *SS* configurations).



Scheme 9

The presence of the chiral centres in the molecules leads to the recording, in their NMR spectra, of different signals for the diastereotopic positions 4 and 6, as well as different signals for axial and equatorial orientations of the atoms at these positions because of the anancomericity of the heterocycle. Both possible diastereoisomers would give the same NMR pattern and the spectral data can not be used to differentiate between them.

The possible *meso* isomer shows an interesting stereochemical aspect because it can generate the stereoisomers A and B, so called "geometrical enantiomers" (Chart 4). This type of molecular asymmetry was first predicted by Shirner e*t al.*,²⁸ than illustrated on two oximes of a piperidone derivative and tropinone respectively.^{29,30}



Chart 4 Geometrical enantiomers of the *meso* diastereoisomer of compounds **21** (R=CH₃), **22** (R=C₂H₅), **23** (R=C₆H₅)

As it can be observed by analyzing their NMR spectra, the bromination reaction of compounds **11** and **12** is also highly diastereoselective, compounds **22** ($R=C_2H_5$) and **23** ($R=C_6H_5$) were obtained as unique diastereomers. Thus, the configuration of the first created chiral centre influences the configuration of the second chiral centre. Compound **21** was obtained as a mixture and the ratio of the diastereoisomers was calculated from 14

the ¹H-NMR spectrum using specific signals intensities and it was estimated to 45:55. The low diastereoselectivity of the dibromination reaction of compound **10** can be correlated to the small volume of the substituent at the chiral centres (methyl for this compound).

The methylene protons at positions β of the ketal part of the 1,3-dioxane ring of compound **22** are diastereotopic and appear, in the ¹H-NMR spectrum, between 1.75 and 2.25 ppm as two complex multiples. The ratio between the values of the integrals corresponding to the two multiples is about 1:3 (one of the protons is more deshielded than the other three) instead of 2:2 as expected. This fact leads us to consider that, in the preferred conformation, one of these protons should be more in the deshielding area of the two oxygen atoms as the other three. Data offered by the 2D Homonuclear and 2D Heteronuclear spectra helped us attribute the deshielded multiplet to the equatorial bromopropyl group.



Figure 2 ¹H-NMR and COSY spectra (fragments) of compound 22

Analyzing the ¹H-NMR spectrum of compound **23** is noteworthy to underline the large value of the diastereotopicity of the protons at positions 4 and 6 ($\Delta\delta_{6ax-4ax}$ =0.62 ppm and $\Delta\delta_{6eq-4eq}$ =0.44 ppm) in comparison with the corresponding values for compound **22**, probably due to the influences exerted by the phenyl groups. The stronger deshielding of the equatorial protons for compound **23** may have the same reason.



Figure 3 ¹H-NMR spectrum (fragment) of compound 23

The new spirane compounds **24-26** exhibit anancomeric structures, as the conformational equilibria of the 1,3-dioxanic ring are shifted towards the conformations displaying the methyl group at position 5 of the heterocycle in equatorial orientation (Scheme 10). The flipping of the carbocycle is frozen due to the two bulky bromine atoms.



Scheme 10

As in the case of the monocyclic dibrominated **21-23**, two new chiral centres are formed in **24-26** and therefore two configurational diastereoisomers are, theoretically, possible: a *meso* isomer (*RS* or *SR* configurations for the chiral carbon atoms) and a racemic *d*,*l* isomer (*RR* or *SS* configurations).

In the syntheses of these spirane derivatives only the *d*,*l* diastereoisomers were obtained, the one with a *"trans"* disposition of the bromine atoms. This conclusion is supported by the NMR spectra, which present only the characteristic signals for one

isomer. Dreiding models investigations of these derivatives show that a *"cis"* disposal of the two bromine atoms would result in a conformationally highly hindered structure.

PART C

SYNTHESIS AND CONFORMATIONAL ANALYSIS OF SOME BIS(1,3-OXATHIANE-2-YL) DERIVATIVES

1. General Aspects Concerning the Stereochemistry of 1,3-Oxathiane Derivatives

1,3-Oxathiane is a very interesting system due to its "schizophrenic" behaviour,³¹ sometimes similar to 1,3-dioxane and other times similar to 1,3-dithiane. Data about its geometry were mainly obtained form ¹H-NMR spectra on different substituted derivatives and in a few cases from X-ray molecular structures. They all confirm that the fundamental conformation is that of a chair with several specifics due to the coexistence of oxygen and sulphur atoms in the same ring.

The stereochemistry of 1,3-oxathiane derivatives^{31,32,33,34,35,36,37} is less studied than the stereochemistry of 1,3-dioxane^{38,39,40} or 1,3-dithiane^{32,41}, mainly due to the relatively difficult access to 3-mercapto-1-propanol synthones and to the complex stereochemistry of the heterocycles bearing different heteroatoms on the ring.

The reported investigations reveal peculiar configurational and conformational aspects. The chirality of the chair conformation of 1,3-oxathiane has been reported, the flipping of the heterocycle results into an enantiomeric interconversion.³¹

This heterocycle – among other saturated six-membered rings with two or more (identical or different) heteroatoms – may be considered as obtained by desymmetrization of adamantane (Scheme 11).⁴²



Scheme 11

The ring-chain tautomerism of 1,3-oxathiane derivatives was investigated by the *cis-trans* isomers equilibria of some spiro-1,3-oxathianes (*e.g.* 9-phenyl-5-oxa-1-thia-spiro[5.5]undecane, Scheme 12) and the kinetic parameters of the reaction were calculated from ¹H-NMR spectra.^{43,36} The equilibration was performed in the presence of residual HCI and water in the CDCI₃ used in recording the ¹H-NMR spectra in matter of hours or in solid state after storing for three weeks on the lab bench.



Scheme 12

The isomerisation involves the opening of the heterocycle to give the open-chain form followed by re-closure of the ring and formation of both diastereoisomers in a ratio determined by the different energies of the two structures. The ring-chain tautomerism is probably reproduced in many substituted 1,3-oxathianes without being observed as the equilibration occurs between homomeric structures with major contribution of the ring form.

2. Synthesis of New Bis(1,3-oxathiane-2-yl) Derivatives

New 1,3-oxathiane derivatives **2-11** were obtained in good to fair yields by the condensation reaction of 2,2-dimethyl-3-mercapto-1-propanol (**1**) with several dicarbonyl compounds (Scheme 13).⁴⁴

The reactions were carried out in acidic conditions (PTSA), in toluene under reflux and the equilibrium was shifted towards the desired product by azeotropic removal of the formed water. The pure compounds were isolated by flash-chromatography using as elution system a mixture of petroleum ether:dichlomethane:ethyl acetate= 10:5:1 (slight modifications of the ratios of the solvents were operated for some of the compounds). Mono-1,3-oxathiane derivatives were observed as side-products in a ratio between 1:1.7 and 1:3 (mono:bis-derivative) as it was measured from the relative intensities of the specific signals in the NMR spectra of crude products for **2-5**. Compounds **9-11** (Scheme 13) were isolated and their molecular structure was confirmed by ¹H-NMR spectra (Table 5). The corresponding *ortho* derivative could not be isolated in pure form.



Scheme 13

2. Structural Aspects in Solution

3.1 Conformational Aspects

All the investigated compounds show anancomeric structures. The conformational equilibria for compounds **2-4** (Scheme 14 - A) and **6** (Scheme 14 - B) are shifted towards the conformers having the substituents at positions 2(2') - the aromatic ring or the second 1,3-oxathiane ring - in equatorial orientation for both heterocycles.





The conformational preference is suggested by the differences of conformational free enthalpies of the alkyl and aryl groups located in the thioacetal part of the 1,3-oxathiane ring^{45,46} and is confirmed by the analyses performed in solution and in solid state. As an example, the ROESY spectrum of compound **6** shows important interactions between the signals corresponding to the axial proton at positions 2 (2') ($\delta_{2(2')ax} = 4.80$ ppm) and the signals belonging to the axial protons at positions 4 (4') and 6 (6') ($\delta_{4(4')ax} = 2.87$ ppm and $\delta_{6(6')ax} = 3.29$ ppm), proving the equatorial disposal of the two 1,3-oxathiane rings.

The conformational equilibria for **5**, **7** and **8** are shifted towards the conformers bearing the aromatic groups located at the thioacetal part of the heterocycles in axial orientation (Scheme 14 - B, respectively Scheme 15 for compound **5**).



Scheme 15 Conformational equilibria for compound 5. Like isomer was chosen for representation.

The preference for the axial disposition of the aromatic ring is in accordance with the experimental spectral data. The ROESY spectrum of compound **5** shows important interactions between the singlet corresponding to the aromatic protons (δ = 7.84 ppm) and the signals belonging to the axial protons at positions 4' (4") and 6' (6") of the heterocycles ($\delta_{4'(4'')ax}$ = 2.58 ppm and $\delta_{6'(6'')ax}$ = 3.30 ppm).

The anancomeric structure of these derivatives in solution is confirmed by the NMR spectra run at *rt* which exhibit different signals for the axial and equatorial protons of the heterocycles and for the axial and equatorial methyl groups located at positions 5 (5'). The equatorial protons at positions 6' and 6" are more deshielded than the axial ones, whereas at positions 4' and 4" the axial protons are the more deshielded ones (Table 5). In some cases the signals belonging to the equatorial protons exhibit a further splitting due to long range couplings (${}^{4}J_{4'(4'')eq-6'(6'')eq}$ =2.5 – 2.7 Hz; see experimental part).

Compound	4 -	٠H	6 -H		5-0	CH ₃
	ax.	eq.	ax.	eq.	ax.	eq.
2	3.13	2.50	3.53	3.75	1.39	0.95
3	3.10	2.46	3.47	3.75	1.33	0.92
4*	2.64	2.01	3.02	3.49	1.23	0.51
5	2.58	2.40	3.30	3.39	1.20	0.82
6	2.87	2.42	3.29	3.61	1.19	0.86
7	2.48	2.33	3.24,	3.26	1.17	0.74
8	2.49	2.33	3.25,	3.27	1.18	0.76
9**	3.09	2.48	3.49	3.78	1.36	0.96
10**	3.11	2.49	3.49	3.79	1.35	0.95
11**	2.55	2.33	3.24	3.37	1.27	0.78

Table 5 Selected NMR data (CD₂Cl₂, δ, ppm) for compounds 2-11

*Spectra run in toluene-d₈, ** Spectra run in chloroform-d

Configurational Aspects

All the investigated compounds are chiral, the chiral elements being the carbon atoms at positions 2' (2") and the virtual tricoordinated chiral centre associated with the chirality of the unsubstituted 1,3-oxathiane rings.

The configuration at C^2 chiral carbon atom of the 1,3-oxathiane ring and the conformational preference of the substituents located at it induce a specific configuration for the virtual chiral centre. As an example for 2-phenyl-1,3-oxathiane due to the equatorial preference of the aromatic group the *S* configuration (*R* configuration) at C-2 determines the *S* configuration (*R* configuration) of the heterocycle in the stable conformer (Scheme 16).



Scheme 16

For these reasons, the configurational aspects for compounds **2-8** are discussed in a simpler manner, taking into account only the configuration of the chiral centres at positions 2' and 2".

The presence in the molecules of two chiral centres – the C-2' and C-2'' carbon atoms – determines the existence of two diastereoisomers. One of them exhibits the same configuration on both chiral centres 2'*R*2"*R*, 2'*S*2"*S* (*like* isomer) and the other one has different configurations of the chiral centres 2'*R*2"*S* (*unlike* isomer).

Despite the presence of two diastereoisomers, NMR spectra of compounds **2-8** run at *rt* (of pure and crude products, too) shows only one set of signals. This is in contradiction with the slight energy differences between the possible *like* and *unlike* isomers, which should result from the synthesis as a mixture with close values of the ratios of the two isomers.

Because there are no reasons for the reactions to occur with such a high diastereoselectivity and to lead only to one stereoisomer, it was assumed that both isomers are formed in the reaction but NMR spectra are not able to differentiate them. In addition, all the attempts to differentiate the possible diastereoisomers by chromatographic methods failed. These NMR and chromatographic data can be explained only by the rapid equilibration (Scheme 17) of *like* and *unlike* diastereoisomers, *via* ring-chain tautomerism (a similar process was already observed for *cis* and *trans* isomers of spiro-1,3-oxathianes)^{36,43} and the recording in the NMR spectra of unique signals for the two diastereoisomers at mean values of the chemical shifts.





Scheme 17

It was considered of interest to run variable temperature NMR experiments in order to observe the modifications of the spectra at low temperatures, meaning separated signals for *like* and *unlike* isomers. It is to expect that the slight structural differences between the two diastereoisomers make difficult their NMR differentiation even if they exhibit "frozen structures" (the equilibration of diastereoisomers should be slow enough at low temperatures).

Despite the very close signals of the two diastereoisomers, in the low temperature spectra of compounds **4**, **7** and **8** some relevant modifications were observed.

Unfortunately, the variable NMR experiments of compounds **3**, **5** and **6** did not show relevant changes.

The ¹H-NMR spectrum of compound **4** run at 270 K (Figure 4 - A) exhibits one singlet for the aromatic protons (δ = 7.43 ppm), one singlet (δ = 5.40 ppm) for the protons at positions 2', 2", two doublets of doublets corresponding to the equatorial protons at positions 6', 6" ($\delta_{6'(6')eq}$ = 3.49 ppm) and 4', 4" ($\delta_{4'(4')eq}$ = 1.80 ppm), two doublets for the axial protons of the same positions ($\delta_{6'(6')ax}$ = 2.92, $\delta_{4'(4')ax}$ = 2.62 ppm) and two singlets for the axial (δ_{ax} = 1.23 ppm) and equatorial (δ_{eq} = 0.51 ppm) methyl groups at positions 5', 5".

The spectrum registered at 190 K (Figure 4- C) exhibit two set of signals with close intensities (3/2) that can be associated with the two diastereoisomers (*like* and *unlike*) of the compound. The differentiation of the signals of the two diastereoisomers ($\delta_{major isomer}$, $\delta_{minor isomer}$) are observed for the signals belonging to the axial protons at positions 2', 2" (**a**: δ = 5.27, 5.26 ppm), 6', 6" (**b**: δ =2.85, 2.84 ppm), 4', 4" (**c**: δ = 2.47, 2.48 ppm) and for the axial methyl groups at positions 5', 5" (δ = 1.32, 1.33 ppm), while for the other protons the signals of the two diastereoisomers remain undifferentiated.



Figure 4 Variable temperature NMR experiments run on compound 4

The ¹H-NMR spectra of compounds **7** and **8** run at low temperature (Figure 5) exhibit an observable multiplication of the initial signals (recorded at *rt*) only for the signal belonging to the protons of the axial methyl groups at positions 5', 5" (**7**, *rt*: δ = 1.17 ppm, 183 K: δ = 1.11, 1.12 ppm; **8**, *rt*: δ = 1.18 ppm, 180 K: δ = 1.10, 1.12 ppm).



Figure 5 Variable temperature NMR experiments run on compound 8

Careful inspection of the spectra recorded at low temperature for **7** and **8** reveals two new sets of signals (with different intensities). These signals are significantly different of those recorded at *rt* and suggest the freezing of the molecules in other conformers than the major chair ones, too. The large geminal coupling constants (up to 16-17 Hz) for the protons of the heterocycles and the very close signals recorded for the methyl groups at positions 5', 5" (isoclinal positions) suggest the presence of 2,5-TB conformations. The two new sets of signals can be associated with the TB conformers of the *like* and *unlike* isomers of the compounds.

Table 6 Selected NMR data (CD₂Cl₂, δ , ppm) for the 2,5-TB conformers of compounds **7** and **8** recorded in low temperature spectra.

Compound	6-	Н	4-	·H	5-CH₃
7	3.52	3.35	2.75	2.26	0.84
1	3.48	3.32	2.71	2.22	0.80
0	3.51	3.34	2.77	2.22	0.83
o	3.48.	3.31	2.71	2.16	0.80

The ratios between chair and twist-boat conformers (measured from the relative intensities of the specific signals in the ¹H-NMR spectra at 180K) are of about 92/8 for **7** and 91/9 for **8**, respectively. From these data a difference of energy between twist boat and chair conformers of **7** and **8** of about 3.30-3.80 kJ / mol was calculated.

As a conclusion, in solution, the investigated 1,3-oxathiane derivatives are implicated in complex configurational and conformational equilibria. These processes involve the equilibration of *like* and *unlike* isomers *via* ring-chain tautomerism and the fast equilibrium between chair and twist-boat conformers, with an observable (in some cases) contribution of non-chair conformers.

3. Structural Aspects in Solid State

The solid state molecular structures of compounds **4** and **5** were determined by X-ray diffractometry. Compound **4** crystallizes as *unlike* isomer. The molecular structure (ORTEP diagram, Figure 6) reveals the chair conformation for both 1,3-oxathiane rings, the equatorial orientation of the aromatic substituent and its preference for the bisectional rotamer [dihedral angle between the plan of the aromatic ring (C^1-C^6) and the best planes of the 1,3-oxathiane rings ($C^1C^2C^5$ or $C^4C^2C^5$) close to 0° (4.46 °)].



Figure 6 ORTEP diagram of compound 4

Compound **5** crystallizes as a "solid solution", the obtained crystals being a mixture of *like* (*RR*, *SS*) and *unlike* (*RS*) isomers. The molecular structure of **5** (Figure 7) reveals the

axial orientation of the aromatic group for both 1,3-oxathiane rings and the preference of the aromatic substituent for the orthogonal rotamer.

An important "disorder" (due to the presence of several isomers in the crystal) at the level of sulphur and oxygen atoms is observed (if these atoms interchange their positions, the configuration of chiral carbon atoms is also changed). The structure could be refined only if specific individual site occupation factors (sof, Table 7) were considered for each of the four locations X^a , X^b , X^c and X^d (X = O or S).



Figure 7 ORTEP diagram of compound 5

Table 7 Individual site occupation factors (sof) for the molecular structure of 5.

Position	Xª	Xp	Xc	Xď
Sof (O/S)	0.543 / 0.457	0.554 / 0.446	0.614 / 0.386	0.423 / 0.577

4. Synthesis of Oxides of Bis(1,3-oxathiane-2-yl) Derivatives

For more structural information, we tried to synthesize the corresponding S-oxides derivatives. We supposed that the newly formed bond will significantly change the magnetic environment in different diastereoisomers leading to major differences in NMR spectra.

There is a limited number of methods for the oxidation of the sulphur atom of 1,3oxathiane derivatives in the literature.^{47,48,49,50} We chose the *m*-chloroperoxybenzoic acid as oxidizing reagent^{47,48} and compounds **4** and **8** were first subjected to this reaction. All our attempts have led to the recovery of the starting 1,3-oxathiane derivatives, despite the variation of main reaction parameters.

The new sulfoxide derivatives **12** and **13** were obtained from the oxidation reaction of compound **6** with 2 equivalents of *m*-chloroperoxybenzoic acid (MCPBA) (Scheme 18).



Scheme 18

In spite of our expectations, NMR spectra of compound **12**, run at *rt*, show only one set of signals, quite similar with the spectra of compound **6**, indicating a symmetrical structure with the same disposal of the oxygen atom at both sulphur atoms.

¹H-NMR spectrum of this compound shows the equatorial protons at positions 4(4') ($\delta_{4(4')eq} = 3.44$ ppm) more deshielded than the axial ones ($\delta_{4(4')ax} = 2.62$ ppm). Introduction of the S-oxide group causes a deshielding of the equatorial protons of about 1.00 ppm compared to the chemical shift of the same protons of the starting compound. This fact, along with the possible 1,3-*syn* axial interactions with the axial methyl group at positions 5, 5', suggests the equatorial disposition of the S \rightarrow O bond in both heterocycles (Chart 5). The equatorial preference of the oxygen in the sulfoxide group was previously observed for 2-methyl-4-propyl-1,3-oxathiane derivative.^{48,49}



Chart 5 Structure of compound 12. Unlike isomer was chosen for representation

PART D – SYNTHESIS OF NEW AMPHIPHILIC PURINE NUCLEOSIDES

1. General Introduction. Scopes and Strategies

Synthesis of nonnatural nucleosides has attracted significant interest over the past 25 years due to the central role that oligonucleotides play in biochemical systems.

Nucleotides, respectively nucleosides are the constituting monomers of nucleic acids. From a chemical point of view, one DNA strand is a "random" copolymer formed by four different nucleotides. Each strand is coupled through H-bonding to a "complementary" one, that is, to another copolymer that contains a complementary base sequence.

Starting from this biochemical background, we were interested in developing combined structures consisting of the normal acid building blocks, *i.e.* nucleobases and ribose or desoxyribose, and lipophilic substituents as long chained alkyl or alkenyl substituents. Such amphiphilic nucleosides reveal interesting supramolecular behaviour. Thus they can arrange as Langmuir-Blodgett films,^{51,52,53,54} liposomes,⁵⁵ vesicles,⁵⁶ gels⁵⁷ and can modify the transport characteristics through lipid membranes. Furthermore, the lipophilic group of amphiphilic nucleosides can anchor in biomembranes⁵⁸ and thus cause hemifusion of giant unilamellar vesicles by pairing of complementary nucleobases.⁵⁹ This ability shall be used to fix functions to the supramolecular structures. Combining amphiphilic nucleobases to the polar side of the membrane. The amphiphilic components float in the membrane and shall be organised in defined assemblies by bringing them into contact with monostranded DNA or PNA. This principle can be used to structure and functionalise such membranes (Figure 8).



Figure 8

Such derivatives have also a number of other possible applications because nucleoside analogues exhibit interesting pharmaceutical and biochemical properties. Antileukemic,^{60,61} immunosuppresive⁵¹ or antitumor agents,^{57,62} kinase inhibitors⁶³, A³ adenosine receptor antagonists⁶⁴ or antiviral products^{62,65} were found in this series. Such compounds could also be incorporated into oligonucleotides.⁶⁶

Of this class of compounds, we focused our interest on the synthesis of lipophilic purine nucleosides which later on allow the formation of oligonucleosides on one hand and exhibit the ability of complementary base coupling on the other hand. Purines bearing carbon substituents in positions 2, 6 and 8 were fairly underdeveloped, due to their limited availability by conventional chemistry. Introduction of a carbon substituent in position 8 of

the purine ring should preserve their base-pairing properties, with the additional substituent pointing into the major groove of DNA, thus influencing triplex formation and/or DNA-protein interactions. Another important feature of the C-C purines is their expected stability towards enzymatic degradation.

Among reported methods, Sonogashira palladium-catalyzed cross-coupling reaction proved to be a robust and versatile C-C coupling reaction, which could also be applied to C-8 substituted purine nucleoside and nucleobases.⁶⁷ Remarkably, protective groups are not necessary in most cases, making this reaction very attractive.^{64,65,68,69,70,71,72} Sonogashira coupling was also applied to provide linkers for groups that allow the application as nucleic acid structural probes.⁷⁰⁻⁷³

2. Synthesis of Some New C-8 Purine Derivatives

Since the C-8 substitution on adenosine and guanosine has been investigated on analogues possessing low lipophilicity, we were interested in developing a study of the synthesis of derivatives bearing long chain alkynyl or alkyl substituents at the position 8 of the heterocycle.

The new compounds **3** - **8** were obtained by the Sonogashira coupling reaction between 8-bromoadenosine **1** or 8-bromo-2'-deoxyadenosine **2** at one side and terminal alkynes with long alkyl chains (commercially available 1-tetradecyne, 1-hexadecyne and 1-octadecyne) at the other side (Scheme 19). Applying the appropriate method, the products were obtained in high yields as crystalline solids or foams.⁷⁴

We chose 8-bromoadenosine as a starting material which can be readily prepared using Ikehara's method, in ca. 70% yield⁷⁵, by direct bromination of commercially available adenosine. For the bromination of 2`-deoxyadenosine, the method described by Ikehara⁷⁶ was improved by stabilizing the pH of the mixture at 5, optimization of the bromine concentration and of the reaction time and temperature. Structure of the compounds **1** and **2** was confirmed by elemental analysis and NMR spectra. These properties were identical with those reported previously.



Scheme 19

The alkynyl group in amphiphilic nucleosides can serve as precursor for a corresponding alkyl group, as could be shown by hydrogenation of compound **5** to the 8-octadodecyl-adenosine **9** (Scheme 20). Thus the sequence of a Sonogashira coupling of nucleosides followed by hydrogenation allows the overall introduction of lipophilic alkyl groups into nucleosides.



Scheme 20

Taking into consideration the obtained results at this stage of our project, we extended the research target by performing the coupling reaction on the guanosine derivatives, too.

In this case, the starting material was 8-bromoguanosine (Scheme 21), product obtained in 70% yield by direct bromination⁷⁷ of the commercially available guanosine.

The first attempts for the cross-coupling reaction were not successful when unprotected 8-bromoguanosine (as for 8-bromoadenosine) was used. Working with the unprotected derivative gave low yields and the brominated starting material or Glasercoupled diynes.

Thus, the synthetic strategy had to include the protection of the hydroxyl groups in the ribose, as well as the protection of the amino group of the heterocycle (Scheme 21). Introduction of TBDMS groups by slightly modified method⁷⁸ in the molecule leads to a decrease in the polarity of the compound, which allowed us the purification of the products by column chromatography on silica. Compounds **11** and **12** are already described in the literature, but the method had to be slightly modified to obtain satisfactory yields. The spectral data of these products agree with the reported ones.⁷⁹





Having the 8-bromo protected guanosine **12** in hand, the utility of this intermediate in palladium-catalyzed cross-coupling reaction was explored in the reaction with 1tetradecyne (Scheme 22). The reaction of bromo compound **12** with the terminal alkyne in the presence of a catalytic amount of $(PPh_3)_2PdCl_2$, Cul proceeded smoothly at *rt* in 85% yield when diisopropylamine was used not only as a base, but also as solvent⁸⁰. The purification of the crude reaction product was afforded by column chromatography on silica using 5% EtOH in CHCl₃ as eluent.



Scheme 22

The structure of the new compound **13** was unambiguously determined by means of NMR and mass spectra (ESI-MS).

The ability to anchor, as well as the localization in membranes of compound **5** has been investigated with regard to nanobiotechnological applications.⁸¹

The investigated lipophilic nucleoside incorporate well into membranes (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine - POPC and 1,2-dimyristoyl-*sn*-glycero-3phosphocholine – DMPC), even at high molar ratio of 20 mol%, without destroying the bilayer structure or the formation of non-lamellar phases. The membrane structure maintained its lamellar liquid-crystalline character, as detected by ³¹P NMR.

The NMR-results of membrane anchored lipophilic nucleoside **5** indicate that the structure of the membrane is hardly disturbed by these hosts as compared with steroidal derivatives. There were registered somewhat broadened ³¹P and ²H-NMR spectra of the membrane. These broadenings could be related to an influence of **5** on the headgroup mobility of the phospholipids.

Besides the stable anchoring of lipophilic nucleoside in membranes, the exposition of the nucleobase in the aqueous phase is important for the recognition and binding to external patterns. If this part of the molecule is not sufficiently hydrophilic, it will be buried in the membrane and resist binding to the external complementary DNA patterns. Therefore, the localization of the nucleobase and ribose moieties with respect to the lipid membranes by ¹H MAS NOESY NMR was also studied.

A preferential localization in the glycerol/headgroup region of the membrane has been found. Due to the accumulation of hydrophilic functional groups, these molecular segments exhibit a propensity for the more polar environment. The interface location of the ribose/nucleobase moiety exposes the adenine to the aqueous phase, where an interaction with external molecular patterns can take place, such as single stranded RNA of DNA, for instance via Watson-Crick base pairing.

3. Attempts on Synthesis of Derivatives Bearing more Lipophilic Substituents

The main target of the project was the preparation of some purine nucleosides bearing lipophilic groups at position 8 of the heterocycle. The next phase involved a study on the synthesis of derivatives bearing more than one lipophilic chain in the substituent at this position.

We considered a synthetic strategy having as key compound the 8-allyl substituted adenosine derivative **16** (Scheme 23).



Scheme 23

For the synthesis of the 8-allyl-derivative **18**, the literature presents only few procedures that could be taken into consideration,^{63,64,82,83,84,85,86} all of them involving reactions with the protected adenosine. Since the next steps for the target molecules would anyway require the protection of the sugar hydroxyl groups we chose to perform the reaction of compound **1** with excess of TBDMS-CI and imidazole (in DMF and THF as co-solvent (5:2) at room temperature for 2-3 days, Scheme 24). It is quite known that the glycosidic bond of purine nucleosides undergo acid-catalyzed hydrolysis, so the TBDMS group met our requirements concerning this problem because of its stability under strongly basic conditions and its easy cleavage under neutral conditions with fluoride anion.

Compound **16** was obtained in 60% yield after column chromatography and the spectral data are similar with those already reported.⁸⁷ We obtained the mono-silylated amino derivative **17** as side-product, which was also fully characterized by means of NMR spectra.



Scheme 24

Having the desired protected bromoadenosine **16**, we than examined the introduction of allyl group at 8 position of the heterocycle. Table 1 presents the results (main products after the flash-chromatography) of the synthesis experiments (Scheme 25). 34

Some of them were repeated several times, changing the reaction time for each step, but no satisfactory results were achieved. The obtained mixture of compound **18** and its isomerisation product **18a** could not be separated by flash-chromatography, because of the similarities of their R_f values in all the considered eluent mixtures. In all reactions, also the starting material **16** was also recovered to some extent.



Scheme 25

The structures of compounds **18**, **18a** and **18b** were determined by means of their NMR spectra.

Table 8

Reagents used	Products mixture		
2 eq. BuLi (-78° C)	20% compound 18 and as side products: 18b and one compound that showed		
0.2 eq. Cul (-35° C)	in NMR spectra the loss of one of the TBDMS groups		
3eq. allyl-bromide (-25° C)			
4 eq. BuLi (-78° C)			
0.2 eq. Cul (-35° C)	About 20 % of the mixture 18 with 18a, and the reduction product 18b		
3eq. allyl-bromide (-25° C)			
5 eq. BuLi (-78° C)	About 20 % of the mixture 18 with 18a , and as side product 19b		
3eq. allyl-bromide (-78° C)	About 20 % of the mixture to with tod, and as side product tob		
5 eq. LDA (-78° C)	Only traces from the desired compound 18 , we isolated approximately 50% of		
3eq. allyl-bromide (-78° C)	the reduction product 18b		
5 eq. (CH ₂ =CH-CH ₃)SnBu ₃	Approximately 30% mixture of 18 with 18a and as side products 18b and		
PdCl ₂ (PPh ₃) ₂ 5 mol%			
Reflux (HMPA)	decomposition products		

We considered the yields of these experiments unsatisfactory and unpractical because the 8-allyl derivative would be only the first step from a multi-step strategy.

The encountered problems and low yields when trying to introduce the allyl substituent at the adenine ring led to a new strategy. Thus, we next examined the synthesis of 8-vinyl adenosine derivative as key intermediate (Scheme 26) for obtaining the target molecules. Using our previous experience, we performed the Sonogashira cross-coupling reaction of the protected protected 8-bromoadenosine with 1,1-dimethyl-propargyl alcohol. We considered more practical to work with the compound **16** in order to avoid the hydroxyl protection step later. Also, the better solubility of this adenosine derivative permitted the use of the base as solvent, too. First, the reaction carried out in diisopropylamine and with $(Ph_3P)_2PdCl_2$ as catalyst led to the recovery of the starting material.

The new compound **21** (Scheme 26) was obtained, by performing the Sonogashira reaction in piperidine with catalytic amount of $(Ph_3P)_4Pd$, in moderate yield, about 40% (unoptimized) after flash-chromatography. The new product gives satisfactory NMR spectra and can be used for the further steps.

This strategy can be continued with the reaction of the new obtained product **21** with KOH and the reduction of the triple bond to the double bond by using Lindlar catalyst in order to obtain the 8-vinyl substituted adenosine derivative for the bis-hydroxylation reaction.



key intermediate - planned to be obtained

Scheme 26

4. Attempts on the Synthesis of Protected 8-Modified-2'-deoxyadenosine Derivatives – for their Use in Automated DNA Synthesis

There is a growing interest in synthetic modified oligonucleotides containing nucleoside-analogues. The incorporation of modified bases into oligonucleotides may produce useful changes in physical and biological properties of the resulting DNA fragments, which can be used as effective for investigation of nucleic acids structures. Non-canonical DNA structures (hairpins, three and four-way junctions, triplexes, etc.)^{88,89} are currently the subject of extensive investigations because of their important biological implications.

In this frame we studied the synthesis of the protected deoxy-adenosine derivatives of compounds **6-8** in order to introduce them into oligonucleotides by means of automated synthesis.

Efficient groups for the protection of exocyclic amino function and hydroxyl groups from the sugar moiety had to be chosen (Chart 6). These groups should allow a gain of time in the preparation of DNA fragments and they have to be stable enough during the assembling steps and easily and totally removable from the oligomer after synthesis, within few hours, in mild reaction conditions. Thus, the phenoxyacetyl (PAC) amine-protecting group was chosen, since it is well-known to undergo deprotection under less harsh conditions, as compared with other known protective groups.⁹⁰ To protect the 5`-hydroxyl of the deoxy-ribose residue, the 4,4`-dimethoxytrityl group (DMT) was preferred, because is more labile in acidic conditions.



Chart 6

6-8 with the phenoxyacetyl group. The experiments results were not satisfactory, there were encountered some problems and it was necessary to change the synthetic strategy by performing first the protection of the 5'-hydroxyl group with the 4,4'-dimethoxytrityl

group. Thus, compound **8** was reacted with 4,4'-dimethoxytrityl chloride (DMT-CI) according to a known procedure (Scheme 27).⁹¹



DMT = 4,4'-dimethoxytrityl

Scheme 27

The new compound **23** was obtained in 60% yield (unoptimized) after column chromatography purification on silica, previously treated with 1% Et_3N , using a 3% EtOH in CHCl₃ eluent mixture.

The structure of this derivative was confirmed by NMR spectra. ¹H-NMR spectrum shows the signals corresponding to the methoxy protons of the newly introduced substituent at 3.72 ppm. The next step will be to perform the protection of the exocyclic amino group of the heterocycle on the new synthesized compound **23**.

6. Acknowledgments

This project was supported by a 12 months fellowship granted by the *Deutscher Akademischer Austauschdienst (DAAD)* and coordinated by Prof. Dr. Juergen Liebscher, Institut für Chemie, Humboldt Universitaet zu Berlin, Berlin, Germany.

My special thanks to Prof. Dr. Juergen Liebscher, the scientific coordinator of this part of the thesis, for entrusting me in this research project and for guiding me in my work.

I also thank Dr. Holger A. Scheidt and Dr. Daniel Huster, Biotechnological-Biomedical Center, University of Leipzig, Leipzig, Germany, Prof. Dr. Andreas Herrmann, Humboldt-University Berlin, Institute of Biology/Biophysics, Berlin, Germany for performing the investigation on the biological activity of the synthesized compounds; Dr. Joachim Leistner for the HPLC analyses. My warm thanks to the technical staff Karola Brosche, Bärbel Brauer and to my colleagues Magda Karanik, Dr. Hans-Juergen Hamann, Christoph Bender, Wolfgang Flasche, Yin Lunxiang, Daniela Schley and Oxana Sieck (Humboldt Universitaet, Berlin, Germany) for advises and help in the laboratory work. I am grateful to the DAAD for the opportunity of this research stage and to the Humboldt University for accepting me as a research associate during the fellowship period.

CONCLUSIONS

1. The complete review of spiro compounds with six-membered rings – carbocycles and oxygen containing heterocyclic spiranes was elaborated. The stereochemical features of these compounds were discussed taking into consideration both configurational and conformational aspects. The synthesis, structure and applications of the main classes of spiranes, classified by the nature of the constitutive six-membered rings were described.

2. The synthesis and stereochemistry of 5 new mono- and 6 new dibrominated 2,5substituted-1,3-dioxane derivatives were discussed. The regio- and stereoselectivity of the bromination reaction, as well as the anancomeric nature of the investigated derivatives were highlighted.

3. The synthesis and stereochemistry of 6 new 1,3-dioxane derivatives, as intermediates for the bromination reaction, were studied.

4. The synthesis of 9 new bis-1,3-oxathiane derivatives (among them two are Soxides) was accomplished. Complex structural analysis by means of X-ray diffraction in solid state (2 structures) and variable NMR experiments in solution have established the anancomeric structures of the derivatives and the conformational preferences of the substituents at position 2 of the heterocycle.

5. The investigations have shown that the *like* and *unlike* structures are in equilibrium through the open-chain forms and the ring-chain tautomerism is very rapid for these derivatives.

6. In case of two of the 1,3-oxathiane compounds, the low temperature NMR spectra have illustrated the significant contribution of the twist-boat conformers (8-9%).

7. 8 New amphiphilic purine nucleosides with lipophilic alkinyl substituents attached to the N-heterocyclic ring were obtained in a straightforward manner by Sonogashira-coupling of 8-bromoadenosine, 2'-deoxy-8-bromoadenosine and protected 8-bromo-guanosine. Hydrogenation of one of the new compounds was also performed.

8. The ability to anchor, as well as the localization in membranes of one of the adenosine derivatives has been investigated with regard to nanobiotechnological applications.

39

9. The study for the introduction of suitable protective groups for automated DNA synthesis was accomplished on the new deoxy-adenosine derivatives.

10. 4 New additional purine derivatives from the (deoxy)ribose series were obtained for further studies.

Selected References:

¹³ E.L. Eliel, V.S. Rao, F.W. Vierhapper, G.Z. Juaristi, W.R. Kenan, *Tetrahedron* **1975**, *4*9, 4339

¹⁴ A. Dennis, M. Delmas, A. Graset, J.P. Gorrichon, *Can. J. Chem.* **1982**, *60*, 1962
 ¹⁵ V.S. Rao, *Can. J. Chem.* **1982**, *60*, 1067

- ¹⁶ G.W. Buchanan, S.H. Preusser, V.L. Webb, *Can. J. Chem.* **1984**, *6*2, 1308
- ¹⁷ T.A. Crabb, M. Porssa, N.F. Elmore, *Magn. Reson. Chem.* **1991**, *29*, 613
- ¹⁸ P. Maroni, J.P. Gorrichon, Bull. Soc. Chim. France 1972, 785
- ¹⁹ C. Giordano, L. Cappi, *J. Org. Chem.* **1992**, *57*, 2765
- ²⁰ E.N. Lawson, W. Kitching, C.H.L. Kennard, K.A. Byriel, *J. Org. Chem.* **1993**, *58*, 2501
- ²¹ S. Mager, M. Horn, I. Grosu, M. Bogdan, *Monatsh. Chem.* **1989**, *120*, 735
- ²² G. Giusti, G. Morales, Bull. Soc. Chim. Fr. 1973, 382
- ²³ G. Castaldi, S. Cavicchioli, C. Giordano, *J. Org. Chem.* **1987**, *5*2, 5642

²⁴ Crina Socaci, I. Grosu, G. Ple, H.W. Zwanziger, E. Mesaros, D. Marginean, S. Mager, *Heterocycl. Commun* **2000**, *6*, 219

²⁵ M.J.O. Anteunis, D. Tavernier, F. Borremans, *Heterocycles* **1976**, *4*, 293 and references mentioned

²⁶ **Crina Cismas**, G. Gabor, I. Grosu, S. Mager, A. Terec, *Heterocycl. Commun.*, in preparation

²⁷ E. Bogdan, I. Grosu, E. Mesaros, L. Toupet, G. Ple, S. Mager, L. Muntean, *J. Chem. Soc. Perkin Trans.* **2000**, 3635
 ²⁸ R.L. Shriner, R. Adams, C.S. Marvel in *Organic Chemistry* **1943**, Volume 1, edited by H.

²⁸ R.L. Shriner, R. Adams, C.S. Marvel in *Organic Chemistry* **1943**, Volume 1, edited by H. Gilman, 2nd edition, John Wiley, New York, 240

²⁹ R.E. Lyle, G.G. Lyle, *J. Org. Chem* **1959**, *24*, 1679

³⁰ H. Singh, B. Razdan, Tetrahedron Lett. **1966**, 3234

³¹ F.G.Riddel, "The Conformational Analysis of Heterocyclic Compounds"; London, Academic Press **1980**, p.113

¹ I. Grosu, S. Mager, E. Mesaros, L. Muntean, **Crina Socaci**, *Studia Univ. "Babes-Bolyai", Chemia* **1997**, *42*, 123

² L. Muntean, T. Gyorgy, **Crina Socaci**, I. Grosu, S. Mager, A. Mihis, *Studia Univ. "Babes-Bolyai", Chemia* **2000**, *45*, 55

³ E. J. Salni, *Chem. Ber.*, **1938**, 1803

⁴ S. Pager, I. Grosu, Studia Univ. "Babes-Bolyai", Chemia **1988**, 33, 47

⁵ I. Grosu, G. Ple, S. Mager, E. Mesaros, A. Dulau, C. Gego, *Tetrahedron* **1998**, *54*, 2905

⁶ M. J. O. Anteunis, D. Tavernier, F. Borremans, *Heterocycles* **1976**, *4*, 293

⁷ A. Terec, L. Muntean, S. Tötös, N. Bogdan, C. Florian, S. Mager, I. Grosu, *Studia Univ. "Babes-Bolyai", Chemia* **2002**, *47*, 194

⁸ L. Muntean, M. Balog, C. Florian, A. Terec, I. Grosu, S. Mager, *Studia Univ. "Babes-Bolyai", Chemia* **2002**, *47*, 201

⁹ K. Pihlaja, J. Jalonen, Org. Mass Spectrom. **1971**, 5, 1363

¹⁰ F. W. Nader, E. L. Eliel, *J. Am. Chem. Soc.* **1970**, *92*, 3050

¹¹ W. F. Bailey, E. L. Eliel, *J. Am. Chem. Soc.* **1974**, *96*, 1798

¹² E.L. Eliel, W.F. Bailey, L.D. Kopp, R.L. Willer, D.M. Grant, R. Bertrand, K.A. Cristensen, O.K. Dalling, M.V. Dutch, E. Wenkert, F.M. Shell, D.W. Cochan, *J. Am. Chem. Soc.* **1975**, *97*, 322

³² E. Kleinpeter in E. Juaristi, *"Conformational Behaviour of Six-membered Rings";* New York, *VCH Publishers* **1995**, p. 201

³³ K. Pihlaja, P. Pasanen, "Oxathiacyclanes: Preparation, Structure and Reactions" in S. Patai: *"The Chemistry of ethers, Crown Ethers, Hydroxyl Groups and Their Sulphur Analogues"*; New York, *suppl E, part 2. Wiley* **1980**, p. 839

³⁴ E. L. Eliel, *Phosphorus Sulphur* **1985**, *24*, 73

³⁵ A. Terec, I. Grosu, G. Ple, L. Muntean, S. Mager, *Heterocycles* 2003, 60, 1477

³⁶ A. Terec, I. Grosu, L. Muntean, L. Toupet, G. Ple, **Crina Socaci**, S. Mager, *Tetrahedron* **2001**, *57*, 8751

³⁷ M. Stuparu, I. Grosu, L. Muntean, G. Ple, **Crina Cismas**, A. Terec, A. Nan, S. Mager, *Monatsh. Chemie* **2004**, *135*, 89

³⁸ M.J.O. Anteunis, D. Tavernier, F. Borremans, *Heterocycles* **1976**, *4*, 293

- ³⁹ E.L. Eliel, "Stereochemistry of Organic Compounds"; New York, Wiley Interscience **1994**, p. 745
- ⁴⁰ E. Kleinpeter, Adv. Heterocycl. Chem. **1998**, 69, 217
- ⁴¹ E.L. Eliel, R.O. Hutchins, *J. Am. Chem. Soc.* **1969**, *91*, 2703
- ⁴² I. Grosu, S. Mager, G. Ple, R. Martinez, *Chirality* **1996**, *8*, 311
- ⁴³ L. Muntean, I. Grosu, S. Mager, G. Ple, M. Balog, *Tetrahedron Lett.* **2000**, *41*, 1967

⁴⁴ Crina Cismas, I. Grosu, G. Ple, E. Condamine, Y. Ramondenc, L. Toupet, I. Silaghi-

Dumitrescu, G. Nemes, A. Terec, L. Muntean, *Structural Chemistry* **2004**, submitted

⁴⁵ P. Pasanen, K. Pihlaja, *Tetrahedron* **1972**, 28, 2617

- ⁴⁶ K. Pihlaja, P. Pasanen, J. Waehaesilta, Org. Magn. Reson. **1979**, *12*, 331
- ⁴⁷ C.J. Palmer, J.E. Casida, *J. Agric. Food Chemistry.* **1995**, *4*3, 498

⁴⁸ W. Deger, M. Gessner, G. Heusinger, G. Singer, A. Mosandl, *J. Chrom.* **1986**, 366, 385

- ⁴⁹ G. Singer, G. Heusinger, A. Mosandl, C. Burschka, *Liebigs Ann. Chem.* **1987**, 451
- ⁵⁰ K. Pihlaja, R. Sillanpaa, M. Dahlquist, G. Stajer, M. Ahlgren, Struct. Chem. **1993**, *4*, 203
- ⁵¹ C. Li, J. Huang, Y. Liang, *Langmuir* **2002**, *17*, 2228
 ⁵² J. Huang, D. Ding, Z. Zhang, B. Shi, Y. Liang, *Synthetic Communications* **1997**, *27*, 681
- ⁵³ C. Li, J. Huang, Y. Liang, *Langmuir* **2000**, *16*, 7701

⁵⁴ R. Perez, F. Pincet, M. Goldmann, C. Mioskowski, L. Lebeau, *Eur. Phys.* J. **1998**, 1

- ⁵⁵ S. Bonaccio, P. Walde, P.L. Luisi, *J. Phys. Chem.* **1994**, *98*, 6661
- ⁵⁶ D. Berti, U. Keiderling, P. Baglioni, Progr. Colloid Polym. Sci. 2002, 120, 64
- ⁵⁷ L.R. Lewis, R.K. Robins, C.C. Cheng, *J. Med. Chem.* **1964**, *7*, 200
 ⁵⁸ F. Pincet, E. Perez, G. Bryant, L. Lebeau, C. Mioskowski, *Phys. Rev. Lett.* **1994**, *73*, 2780
- ⁵⁹ F. Pincet, L. Lebeau, S.J. Cribier, *Eur. Biophys.* **2001**, *30*, 91
- ⁶⁰ D.T. Warner, G.L. Neil, A.J. Taylor, W.J. Wechter, *J. Med. Chem.* **1972**, *15*, 790

⁶¹ S. Shuto, H. Itoh, S. Ueda, S. Imamura, K. Fukukawa, M. Tsujino, A. Matsuda, T. Ueda, *Chem. Pharm. Bull.* **1988**, *36*, 209

⁶² W.J. Wechter, M.A. Johnson, C.M. Hall, D.T. Warner, A.E. Berger, A.H. Wenzel, D.T. Gish, G.L. Neil, *J. Med. Chem.* **1975**, *18*, 339

⁶³ S. Manfredini, P.G. Baralde, E. Durini, L. Porcu, A. Angusti, S. Vertuani, N. Solaroli, E. De Clercq, A. Karlsson, J. Balzarini, *J.Bioorg. Med. Chem. Lett.* **2001**, *11*, 1329

⁶⁴ R. Volpini, S. Costanzi, C. Lambertucci, S. Vittori, K.-N. Klotz, A. Lorenzen, G. Cristalli, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1931

⁶⁵ G. Sági, L. Ötvös, S. Ikeda, G. Andrei, R. Snoeck, E. De Clercq, *J. Med. Chem.* **1994**, 37, 1307

⁶⁶ E.A. Lesnik, C.J. Guinosso, A.M. Kawasaki, H. Sasmor, M. Zounes, L.L. Cummins, D.J. Ecker, P.D. Cook, S.M. Freier, *Biochemistry* **1993**, *32*, 7832

⁶⁷ L.A. Agrofoglio, I. Gillaizeau, Y. Saito, *Chem. Rev.* **2003**, *103*, 1875

⁶⁸ D. Loakes, D.M. Brown, S.A. Salisbury, M.G. McDougall, C. Neagu, S. Nampalli, S. Kumar, *Tetrahedron Letters* **2003**, *44*, 3387

⁶⁹ C. McGuigan, C.J. Yarnold, G. Jones, S. Velázquez, H. Barucki, A. Brancale, G. Andrei, R. Snoeck, E. De Clercq, J. Balzarini, *J. Med. Chem.* **1999**, *42*, 4480

- ⁷⁰ C. McGuigan, H. Barucki, S. Blewett, A. Carangio, J.T. Erichsen, G. Andrei, R. Snoeck, E. De Clercq, J. Balzarini, *J. Med. Chem.* **2000**, *43*, 4993
- ⁷¹ C.M. McKeen, L.J. Brown, J.T.G. Nicol, J.M. Mellor, T. Brown, *Org. Biomol. Chem.* **2003**, *1*, 2267
- ⁷² J.D. Kahl, M.M. Greenberg, *J. Am. Chem. Soc.* **1999**, *121*, 598
- ⁷³ C. McGuigan, A. Brancale, G. Andrei, R. Snoeck, E. De Clercq, J. Balzarini, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4511
- ⁷⁴W. Flasche, Crina Cismas, A. Herrmann, J. Liebscher, Synthesis 2004, in print
 ⁷⁵M. Ikehara, M. Kaneko, *Tetrahedron.* 1970, *26*, 4251
- ⁷⁶M. Ikehara, M. Kaneko, *Chem. Pharm. Bull.* **1970**, *18*, 2441
- ⁷⁷ a). M. Ikehara, K. Muneyama, *Chem. Pharm. Bull.* **1965**, *13*, 639; b). C. Sheu, C.S. Foote, *J. Am. Chem. Soc.* **1995**, *117*, 6439
- ⁷⁸ C. Sheu, P. Kang, S. Khan, C.S. Foote, *J. Am. Chem. Soc.* **2002**, *124*, 3905
- ⁷⁹ a). J.L. Sessler, R. Wang, *J. Org. Chem.* **1998**, *63*, 4079; b). D. Graham, J.A. Parkinson, T. Brown, *J. Chem. Soc. Perkin Trans. 1*, **1998**, 1131
- ⁸⁰ J.L. Sessler, M. Sathiosatham, C.T. Brown, T. A. Rhodes, G. Wiederrecht, *J. Am. Chem. Soc.* **2001**, *123*, 3655
- ⁸¹H.A. Scheidt, W. Flasche, **Crina Cismaş**, M. Rost, A. Herrmann, J. Liebscher, D. Huster, *J. Phys. Chem.* **2004**, accepted
- ⁸²M. Abarbri, J. Thibonnet, L.Berillon, F. Dehmel, M. Rottlander, P. Knochel, *J. Org. Chem.* **2000**, *65*, 4618
- ⁸³R. Moriarty, W.R. Epa, A.K.A. Awasthi, *Tetrahedron Lett.* **1990**, *31*, 5877
- ⁸⁴ M. Abarbri, F. Dehmel, P. Knochel, *Tetrahedron Letters* **1999**, *40*, 7449
- ⁸⁵ C. Chassaing, A. Haudrechy, Y. Langlois, Synth. Commun. 1997, 27, 61
- ⁸⁶ a). H. Hayakawa, K. Haraguchi, H. Tanaka, T. Miyasaka, *Chem. Pharm. Bull.* 1987, 35, 72 b). K. Haraguchi, T. Saitoh, F. Takai, T. Miyasaka, *J. Heterocycl. Chem.* 1989, 26, 189
 ⁸⁷ E. Halbfinger, D.T. Major, M. Ritzman, J. Ubl, G. Reiser, J.L. Boyer, K.T. Harden, B.
- Fischer, *J. Med. Chem.* **1999**, *42*, 5325 ⁸⁸ J. Feigon, K. M. Koshlap, F. W. Smith in *Methods in Enzymology*; T. L. James, Ed.
- Academic: San Diego **1995**, *261*, 225
- ⁸⁹ R. H. Shafer, Prog. *Nucleic Acids Res. Mol. Biol.* **1998**, *50*, 55
- ⁹⁰ J.C. Schulhof, D. Molko, R. Teoule, Nucleic Acids Research **1987**, *15*, 397
- ⁹¹ D. Graham, J.A. Parkinson, T. Brown, *J. Chem. Soc., Perkin Trans.* 1 **1998**, 1131