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Synthesis of 5'-*O*- and 3'-*O*- Nucleoside Carbamates.

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Abstract: The two step synthesis of 5'-*O*- and 3'-*O*-(*N*-alkylcarbamoyl)-nucleosides is described. The key step is the enzymatic synthesis of 5'-*O*- or 3'-*O*-alkoxycarbonylnucleoside in a regioselective way. In a second aminolysis step these carbonates yield the corresponding urethanes. This methodology allows to assemble aminoalcohols and L-amino acids to 2'-deoxynucleosides.

INTRODUCTION

Although several chemical techniques are available for the regioselective modification of the nucleoside carbohydrates, the development of enzymatic processes for this purpose has been progressing steadly.[1]

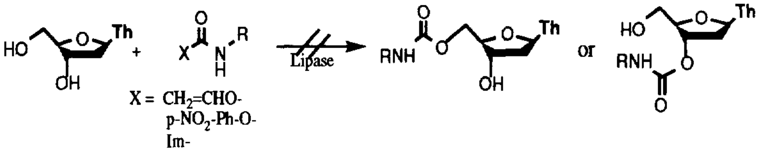
So, significant advantages can be achieved by using enzymatic instead of chemical methods as far as regioselectivity and time saving are concerned. For instance, highly regiodiscriminating acylations of alcohol groups of several nucleosides have been carried out employing the protease Subtilisin with dimethylformamide as solvent.[2] In our group, regioselective acylations[3] and alkoxycarbonylations[4] have been achieved by means of two complementary lipases which allow us to discern among the hydroxyl groups of the nucleoside.

Bearing all this in mind and taking into account the interesting features showed by the carbamate moiety in the nucleoside field, such us phosphate simulation ability[5] and chemical and enzymatic stability, we believed interesting to propose a path which allowed to synthesize the mentioned nucleoside carbamates through a simple and general procedure.[6]

RESULTS AND DISCUSSION

Some 5'-*O*-carbamoylnucleosides[7] and oligonucleosides linked by carbamate backbones[8] have been previously obtained by means of methodologies which make use of highly reactive compounds like phosgene, chloroformates, isocyanates, etc. These procedures require time consuming protection and deprotection steps due to the occurrence of various hydroxyl groups in the sugar skeleton of the nucleoside. That is the chief reason why we thought of taking advantage of the ability of enzymes in discerning one out of several reactive positions, in order to obtain in a direct and regioselective way the corresponding 5'- and 3'-carbamates.

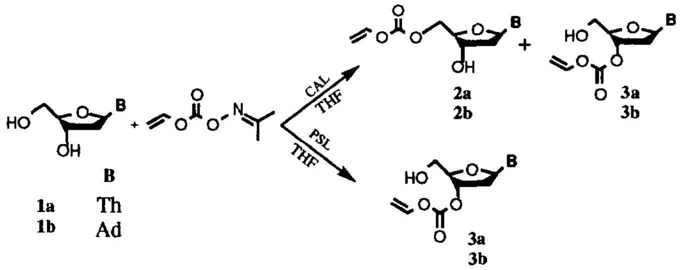
Synthesis of 5'-O- and 3'-O-(N-alkylcarbamoyl)nucleosides. The fastest way, which we thought of, was the direct introduction of the carbamoyl moiety through a reaction between the nucleoside and the proper carbamate catalyzed by a lipase (see Scheme 1). Thus, we initially tested the enzymatic reaction between several carbamoylation agents and thymidine in different organic solvents. Nonetheless, we never attained satisfactory results when CAL, PSL, CCL and PPL were assayed, either because sometimes the products were not formed or because the reaction proceeded in absence of the enzyme yielding several products without selectivity. One feasible explanation to the lack of enzymatic catalysis might be the fact that some carbamates have been shown to be good inhibitors of many serine hydrolases.[9]



SCHEME 1

Once the one-step procedure had to be abandoned, we thought about a possible alternative procedure which involved the regioselective synthesis of an alkoxycarbonylated nucleoside and further reaction of this intermediate with an amine. Therefore, we attempted the reaction between 5'- and 3'-methyl, allyl, benzyl, vinyl and acetonoxime carbonates of thymidine[4] and butylamine in THF at 60°C. Only when the vinyl and acetonoxime carbonates were used the products were obtained.

Hence,the key step was the enzymatic reaction to obtain the corresponding carbonates in a regioselective manner. Because of this, the conditions were optimized with the aim of improving the regioselectivity-yield relationship. It must be pointed out that we can direct the regioselectivity in this first step by changing the lipase used to catalyze the process, as we can see in Scheme 2. So, we could steer the vinyloxycarbonylation step toward the 3' hydroxyl group with lipase from *Pseudomonas cepacia* , without formation of the other regioisomer. Only small quantities of the 5',3'-divinyloxycarbonylated products were detected. Since PSL became inactivated after 24 h, a second addittion of the enzyme was performed. On the other hand, *Candida antarctica* lipase afforded as the main product the 5' carbonate, and small amounts of the other regioisomer (see Table 1). In any case, the products were easily isolated by flash chromatography.

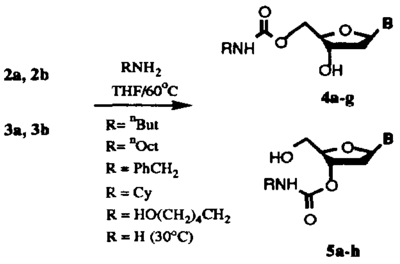


SCHEME 2

Table 1. Enzymatic Synthesis of 5'-*O* and 3'-*O*-(vinyloxycarbonyl)-2'-deoxynucleosides.

B	Enzyme	T(°C)	t(h)	Solvent	Yield(%)	Rate (product)
Th	CAL	30	24	THF	85	8(**2a**):2(**3a**)
Ad	CAL	30	24	THF	82	9(**2b**):1(**3b**)
Th	PSL	30	60	THF	80	**3a**
Ad	PSL	30	60	THF	52	**3b**

Moreover, the aminolysis reaction proceeded in quantitative yields (see Table 2 and Scheme 3) and it allowed us to bind a wide range of alkyl and hydroxyalkylamines to 2'-deoxynucleosides. The latter are interesting compounds, since they can be used not only as precursors of other nucleoside derivatives, but also for linking nucleosides to polymer supports for performing the synthesis of oligonucleotide analogs. The ammonolysis reactions (R=H, Scheme 3) were carried out at 30°C and the products obtained without purification steps.



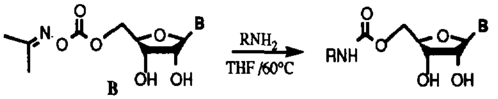
SCHEME 3

Table 2. Compounds **4a-g**, **5a-h** and **7a-b**.[a]

Product	B	R	Yield (%)[b]	mp(°C)[c]	IR[d]	Mass spectra[e, f]
4a	Th	^{n}But	80	202	1691	341(M^+,1), 216(12), 81(100)
4b	Th	$PhCH_2$	92	211	1701	250(M^+-125,14), 133(80)
4c	Th	Cy	83	181	1693	367(M^+,1), 242(16), 117(38), 81(100)
4d	Th	$(CH_2)_5$-OH	87	127	1694	246(M^+-125,8), 125(40), 117(100), 81(95)
4e	Ad	^{n}Oct	86	sirup	1705	406(M^+,1), 251(2), 162(40), 134(100)
4f	Ad	H	99	110	1691	294(M^+,1), 251(2), 221(5), 162(30), 135(100)
4g	Ad	$(CH_2)_5$-OH	86	109	1691	251(M^+-129,3), 221(7), 162(40), 134(100)
5a	Th	^{n}But	80	180	1699	216(M^+-125,18), 118(90), 69(90)
5b	Th	^{n}Oct	80	163	1714	272(M^+-125,5), 174(40), 99(100)
5c	Th	Cy	84	182	1691	367(M^+, 0.5), 242(10), 144(100)
5d	Th	H	99	216	1707	285(M^+,1), 242(2), 160(20), 126(50), 99(85), 69(100)
5e	Th	$(CH_2)_5$-OH	90	133	1710	371(M^+,1), 246(20), 148(40), 69(100)
5f	Ad	$PhCH_2$	86	229	1691	250(M^+-134,2), 221(6), 162(22),134(100)
5g	Ad	H	99	190	1691	294(M^+,1), 251(3), 221(5), 162(30), 134(100)
5h	Ad	$(CH_2)_5$-OH	92	183	1692	246(M^+-134,1), 221(4), 162(40), 134(100)
7a	U	$PhCH_2$	75	135	1697	377(M^+,10), 266(35), 150(40), 91(100)
7b	Ad	^{n}Oct	70	176	1699	422(M^+,30), 288(20), 164(70), 99(100)

[a] All reactions carried out at 60°C except that for **4f**, **5d** and **5g** (30°C). [b] Calculated with respect to the starting carbonates **2a-b**, **3a-b** or **6a-b**, on pure isolated products. [c] Uncorrected. [d] KBr, partial. [e] EI, 70 eV. [f] Satisfactory microanalysis obtained: C, H, N ± 0.3.

As far as ribonucleosides are concerned, carbamoylation in the 5'-*O* position resulted in very good yields, since, as we have previously described the enzymatic formation of the 5'-*O*-alkoxycarbonylated intermediates proceeded in a regioselective and almost quantitative manner.[4a] It must be mentioned that when this step is performed using *O*-(vinyloxycarbonyl)-oxime in presence of CAL, both the vinyloxy and the acetonoxime group can function as leaving group, and then two possible 5' carbonates are formed. So, we verified that the same procedure could be applied with the carbonates **6a-b** (Scheme 4, Table 2).



B
6a U
6b Ad

7a-b

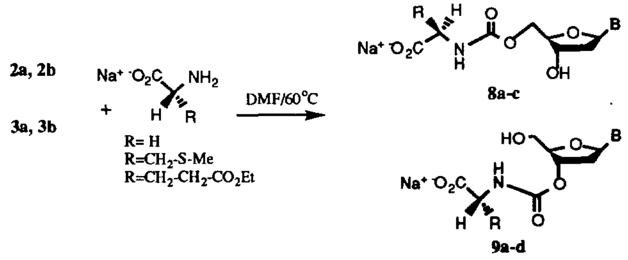
SCHEME 4

Synthesis of N*-[5'- and 3'-(2'-deoxynucleosidyl)oxycarbonyl]-*L*-amino acids (8a-c, 9a-c).* Amino acid linked to nucleosides are an important kind of products in biology, since they have been found to occur in many transfer ribonucleic acids (tRNA).[10] Related compounds have many promising features. They may be used as therapeutic agents as well as being of value for the study of genetic mechanisms. Due to this, we extended the aforementioned methodology to synthesize some amino acid assembled to nucleoside analogs.

However, some problems were found when trying to apply the same procedure with amino acids instead of amines, probably due to their low solubility in organic solvents such as THF. So, we initially attempted the reaction between the vinyloxycarbonylated 2'-deoxynucleosides **2a,b** and **3a,b** and the L-amino acid methyl or ethyl esters in THF, Py and DMF. When using serine, histidine and phenylalanine esters the reactions were very slow and took place with formation of appreciable amounts of the hydrolysis products. Only glycine ethyl ester gave satisfactory results (60% yield).

Then we carried out the reaction with the unprotected L-amino acids. Once again the reactions performed in Py and DMF did not give good results at temperatures varying from 30 to 80°C. As the main problem seemed to be the low solubility of the amino acid, we tried to overcome this problem by using Dioxane/water[11] and two phase ($AcOEt:H_2O$) basic systems (NaOH 0.1M, pH= 9-10), in the last case employing tetrabutylamonium chloride as phase transfer catalyst. Even when the reaction yielded the desired products, appreciable amounts of hydrolysis products were also formed.

The best results were obtained submitting the corresponding vinyloxycarbonylated 2'-deoxynucleosides to reaction with the amino acid sodium salt in DMF at 60°C (Scheme 5). Reactions proceeded fast and without formation of measurable quantities of hydrolysis products. Generally, sonication of the starting materials was performed in order to dissolve the amino acid salts faster. Reactions were stopped when starting vinylcarbonates disappeared, and products were purified by flash chromatography.



SCHEME 5

Table 3. Compounds **8a-c** and **9a-d.**[a]

Product	B	R	Yield (%)[b]	mp (°C)[c]	IR[d]	$[\alpha]^{D}_{25}$ (c, solvent)
8a	Th	H	60	236	1722,1670,1584	+8.5 (0.40, AcOH)
8b	Ad	H	75	227	1707,1649,1597	-6.0 (0.50, AcOH)
8c	Ad	CH_2SMe	85	234	1699,1645,1602	-12.3 (0.55, AcOH)
9a	Th	H	75	218	1697,1680,1579	+4.5 (0.44, AcOH)
9b	Th	CH_2SMe	87	239	1707,1675,1604	-11.2 (0.49, AcOH)
9c	Th	$(CH_2)_2CO_2Et$	70	183	1707,1596	-3.56 (0.87, AcOH)
9d	Ad	$(CH_2)_2CO_2Et$	78	205	1712,1651,1599	-27.6 (1.45, AcOH)

[a] All reactions carried out at 60°C. [b] Calculated with respect to the starting carbonate **2a-b** and **3a-b** on pure isolated products. [c] Products decompose at 135-140°C. [d] KBr, partial.

All the above mentioned carbamates were determined by means of their spectral data. With regard to the ^{1}H-NMR, the more significant change occurs as a downfield shift of *ca.* 0.5 ppm. on the H5′ of the 5′-carbamates (or *ca.* 0.9 ppm. on the H3′ of the 3′-carbamates) with respect to the nucleosides without modification. Some signals were assigned using selective $^{3}J_{H\text{-}H}$ homodecoupling experiments. In addittion ^{13}C-NMR spectra presents a shift of *ca.* 3 ppm. on the C5′of the 5′-carbamates and of *ca.* 4 ppm.on the C3′of the 3′-carbamates towards lower fields again. C1′ and C4′ were assigned on the basis of the coupled ^{13}C-NMR spectra, being the $^{1}J_{C1',H\text{-}C1'}$ values close to 170 Hz while the other $^{1}J_{C\text{-}H}$ of the sugar moiety are about 15 Hz smaller.[14] Complete ^{13}C- and ^{1}H-NMR spectral data are given in Tables 4, 5, 6 and 7 (Experimental).

CONCLUSION

This work shows how enzymes can be exploited to discern between several similar reactive positions. Bearing this idea in mind, we have developed a methodology which allow to attain nucleoside analogs in a two step procedure and with high regioselectivity and yield.

EXPERIMENTAL

Lipase from *Candida antarctica* SP 435L was gifted by Novo Nordisk Company. Amano PS lipase was purchased from Amano Pharmaceutical Co., and those from porcine pancreas (PPL) and *Candida cylindracea* (CCL) were purchased from Sigma. Nucleosides, amino acids and amines were purchased from Aldrich Chemie and Sigma. THF was distilled over $LiAlH_4$, DMF over P_2O_5 and Py over KOH and they were stored under nitrogen atmosphere in order to avoid moisture. Precoated TLC alumina sheets silica gel 60 F_{254} from Merck were used, and for column chromatography, Merck silica gel 60/230-400 mesh was used. Melting points were taken on samples in open capillary tubes using a Büchi melting point apparatus and they are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. IR spectra were obtained on a Perkin-Elmer 1720-X FT spectrometer. NMR spectra were recorded using a Brucker AC300 spectrometer. Deuterated solvents were purchased from Merck. Mass spectra were obtained on a Hewlett-Packard 5897 A spectrometer. Fast-atom bombardment (FAB) mass spectra were recorded on a Finnigan-MAT mass spectrometer Model MAT 95 operating with a Personal Dec Station 5000/33 and an Ion Tech FAB Cesium Gun working at 20 Kv. Microanalyses were performed on a Perkin-Elmer model 240B analyzer. Sonications were performed using a Branson Sonifier 450. Acetone *O*-(vinyloxycarbonyl)-oxime was prepared in almost quantitative yield by treating acetone oxime with vinylchloroformate and distilling under vacuum. Amino acid sodium salts were prepared by solving the correspondig L-amino acids in H_2O, adjusting the pH to 10-11 with NaOH(1N) and evaporating under vacuum. The L-Glutamic acid 5-ethyl ester becomes hydrolysed under the former conditions. Thus, its sodium salt was obtained by treating the amino acid with equivalent amounts of NaOH in MeOH, filtrating and evaporating.

*General procedure for the synthesis of compounds **2a-b**:* 1 mmol of **1a-b**, 4 mmol of *O*-(vinyloxycarbonyl)-oxime and 0.2g of lipase from *Candida antarctica* SP435L were suspended in 20 ml of THF (in the case of **1b**, 0.2g of molecular sieve activated powder were added to remove hydrated water from starting nucleoside). The mixture was allowed to react at 30°C and 250 rpm during the time indicated in the Table 1. Then, the enzyme was filtered off and washed with MeOH, the residue was evaporated under vacuum and the product subjected to flash chromatography (AcOEt in the case of **2a** and AcOEt-MeOH 93:7 for **2b**). Compound **2a** was previously described by us.[4a]

Compound **2b**: mp 73°C; IR (KBr, cm^{-1}) 1763; $[\alpha]^{D}_{25}$= -22.89 (c=0.8, MeOH); ^1H-NMR (DMSO-d_6) δ: 8.35 (1H, s, H8), 8.18 (1H, s, H2), 7.32 (2H, br, NH_2), 7.08 (1H, dd, CH), 6.38 (1H, t, H1'), 5.55 (1H, d, 3'-OH), 4.93 (1H, dd, CH_2), 4.68 (1H, dd, CH_2), 4.52(1H, m, H3'), 4.40 (2H, m, H5', H5''), 4.05 (1H, m, H4'), 2.88 (1H, m, H2'), 2.37 (1H, m, H2''); ^{13}C-NMR (DMSO-d_6) δ: 156.12 (C6), 152.67 (C2), 151.98 (C=O),149.10 (C4), 142.81 (CH),139.58 (C8), 119.24 (C5),98.54 (CH_2), 83.72 (C4'), 83.53 (C1'), 70.59 (C3'), 68.22 (C5'), 38.52 (C2'); Mass spectra (70ev) m/z,%: 321 (M^+,2), 234 (4), 164 (40), 135 (100). Anal. Calcd for $C_{13}H_{15}N_5O_5$: H, 4.71; C, 48.60; N, 21.80. Found: H, 4.75; C, 48.55; N, 21.87.

General procedure for the synthesis of compounds ***3a-b***: 1 mmol of **1a-b**, 1.5 mmol of *O*-(vinyloxycarbonyl)-oxime and 0.25g of lipase Amano PS were suspended in 10 ml of THF under nitrogen atmosphere. The mixture was allowed to react at 30°C and 250 rpm. 2 mmol of *O*-(vinyloxycarbonyl)-oxime and 0.25 g of lipase Amano PS more were added after 24 h. The mixture was allowed to react during the time indicated in the Table 1. Then, the enzyme was filtered off and washed with MeOH, the residue was evaporated under vacuum and subjected to flash chromatography (AcOEt in the case of **3a** and AcOEt-MeOH 93:7 for **3b**). Compound **3a** was previously described by us.[4b]

Compound **3b**: mp 160°C; IR (KBr, cm^{-1}) 1759; $[\alpha]^{D}_{25}$= -42.64 (c=0.53, MeOH); ^{1}H-NMR (DMSO-*d*$_6$) δ: 8.37 (1H, s, H8), 8.14 (1H, s, H2), 7.45 (2H, br, NH_2), 7.09 (1H, dd, CH), 6.38 (1H, t, H1'), 5.62 (1H, d, 5'-OH), 5.43 (1H, d, H3'), 4.97 (1H, dd, CH_2), 4.72 (1H, dd, CH_2), 4.23 (1H, m, H4'), 3.65 (2H, m, H5', H5''), 3.05 (1H, m, H2'), 2.63 (1H, m, H2''); ^{13}C-NMR (DMSO-*d*$_6$) δ: 156.36 (C6), 152.61 (C2), 151.57 (C=O), 148.98 (C4), 142.93 (CH), 139.87 (C8), 119.48 (C5), 98.77 (CH_2), 85.02 (C4'), 84.31 (C1'), 80.31 (C3'), 61.88 (C5'), 36.36 (C2'); Mass spectra (70ev) m/z,%: 321 (M^+,3), 234 (10), 134 (100). Anal. Calcd for $C_{13}H_{15}N_5O_5$: H, 4.71; C, 48.60; N, 21.80. Found: H, 4.72; C, 48.59; N, 21.84.

General procedure for the synthesis of compounds ***4a-g, 5a-h*** *and* ***7a-b***: 1 mmol of the carbonate **2a-b**, **3a-b** or **6a-b** and 2.5 mmol of the corresponding amine were suspended in 10 ml of THF and allowed to react at 60°C and 250 rpm under N_2 (in the case of the ammonolysis reactions were performed at 30°C in dioxane saturated of NH_3). The reactions were stopped when TLC shows no more starting material remaining in solution (1-2 days). Then, the solvent was evaporated under vacuum and the residue subjected to flash chromatography using the following eluents: AcOEt-MeOH 95:5 except for **5a-c** (AcOEt), **4d**, **5e**, **7a**,**7b** (AcOEt-MeOH 9:1) and **4g**, **5h** (AcOEt-MeOH 7:3). In the reactions with NH_3, it was not necessary to perform chromatography purification.

General procedure for the synthesis of compounds ***8a-c*** *and* ***9a-d***: 1 mmol of **2a-b** or **3a-b** and 2 mmol of the amino acid sodium salt were suspended in 20 ml of DMF (the solution was sonicated for 2 min in order to dissolve faster the amino acid). 0.2g of molecular sieve activated powder were added and the mixture was stirred at 60°C till there was no more starting carbonate remaining in solution (generally less than one day). Then, solvent was evaporated under vacuum and the residue subjected to flash chromatography (AcOEt -MeOH 6:4). Products were then dissolved in water, the solution carefully adjusted to pH=7 with 0.01N NaOH and then they were well dried. Products were sometimes washed with MeOH.

Characterization of Products ***4a-g***, ***5a-h***, ***7a-b***, ***8a-c*** *and* ***9a-d***: Table 2 shows yield, mp, IR data and mass spectra of **4a-g**, **5a-h** and **7a-b** while table 3 shows yield, mp, IR data and optical rotations of compounds **8a-c** and **9a-d**. Tables 4, 5, 6 and 7 present the ^{1}H- and ^{13}C-NMR spectral data and solvents used in their measurement.

8a: mass spectra *m/z* (FAB+, relative intensity) 366 (M+ H, 20)+; 388 (M+ Na, 10)+; 115 (100). Anal. calcd. for $C_{13}H_{16}N_3O_8Na$: H, 4.42; C, 42.75; N, 11.50. Found: H, 4.44; C, 42.82; N, 11.36.

8b: mass spectra *m/z* (FAB$^+$, relative intensity) 375 (M+ H, 10)$^+$; 397 (M+ Na, 50)$^+$; 115 (100). Anal. calcd. for $C_{13}H_{15}N_6O_6Na$: H, 4.04; C, 41.70; N, 22.46. Found: H, 4.10; C, 41.78; N, 22.29.

8c: mass spectra *m/z* (FAB$^+$, relative intensity) 435 (M+ H, 10)$^+$; 457 (M+ Na, 30)$^+$; 115 (100); *m/z* (FAB$^-$, relative intensity) 433 (M- H, 70)$^-$; 411 (M - Na, 30)$^-$; 160 (100). Anal. calcd. for $C_{15}H_{19}N_6O_6SNa$: H, 4.41; C, 41.47; N, 19.35. Found: H, 4.37; C, 41.49; N, 19.34.

9a: mass spectra *m/z* (FAB$^+$, relative intensity) 366 (M+ H, 30)$^+$; 388 (M+ Na, 30)$^+$; 115 (100); *m/z* (FAB$^-$, relative intensity) 364 (M- H, 10)$^-$; 131 (100). Anal. calcd. for $C_{13}H_{16}N_3O_8Na$: H, 4.42; C, 42.75; N, 11.50. Found: H, 4.47; C, 42.80; N, 11.41.**9b**: mass spectra *m/z* (FAB$^+$, relative intensity) 426 (M+ H, 10)$^+$; 448 (M+ Na, 20)$^+$; 115 (100); *m/z* (FAB$^-$, relative intensity) 424 (M- H, 70)$^-$; 160 (100). Anal. calcd. for $C_{15}H_{20}N_3O_8SNa$: H, 4.74; C, 42.34; N, 9.88. Found: H, 4.80; C, 42.37; N, 9.82.

9c: mass spectra *m/z* (FAB$^+$, relative intensity) 466 (M+ H, 50)$^+$; 488 (M+ Na, 60)$^+$; 115 (100); *m/z* (FAB$^-$, relative intensity) 464 (M- H, 20)$^-$; 199 (100). Anal. calcd. for $C_{18}H_{24}N_3O_{10}Na$: H, 5.20; C, 46.44; N, 9.03. Found: H, 5.23; C, 46.37; N, 8.96.

9d: mass spectra *m/z* (FAB$^+$, relative intensity) 475 (M+ H, 20)$^+$; 497 (M+ Na, 10)$^+$; 115 (100). Anal. calcd for $C_{18}H_{23}N_6O_8Na$: H, 4.89; C, 45.56; N, 17.72. Found: H, 4.91; C, 45.53; N, 17.79.

Table 4. ^{1}H-NMR Spectral data for Compounds **4a-g**, **5a-h** and **7a-b** (only non-interchangable signals) δ(ppm).[a]

Product	Base ring		Sugar moiety					Carbamate moiety
	Me(H8)	H6(H2)	H1'	(H2',H2'')	H3'	H4'	(H5',H5'')	
4a	1.77(s)	7.43(s)	6.19(t)	2.15(m) 1.95(m)	4.17(m)	3.91(m)	4.05(m)	2.96(m,2H); 1.30(m,4H); 0.84(t,3H)
4b	1.72(s)	7.44(s)	6.22(t)	2.20(m) 2.08(m)	4.18[b]	3.91(m)	4.11(m)	4.19[b]; 7.26(m,5H)
4c	1.74[b]	7.45(s)	6.20(t)	2.19(m) 2.05(m)	4.22(m)	3.89(m)	4.09(m)	3.22(m,1H); 1.79[b];1.15(m,5H)
4d	1.77(s)	7.43(s)	6.18(t)	2.18(m) 2.08(m)	4.19(m)	3.90(m)	4.08(m)	2.97(m,2H); 1.36(m,6H);3.40(m,2H)
4e	8.48(s)	8.37(s)	6.62(t)	2.95(m) 2.65(m)	4.75(m)	4.35(m)	4.52(m)	3.25 (t,2H); 1.65(m,2H); 1.43(m,10H); 1.05(t,3H)
4f	8.37(s)	8.12(s)	6.37(t)	2.85(m) 2.31(m)	4.42(m)	4.01(m)	4.15(m)	
4g	8.47(s)	8.33(s)	6.61(t)	3.00(m) 2.67(m)	4.77(m	4.33(m)	4.52(m)	3.27(t,2H); 1.77(m,6H); 3.77(t,2H)
5a	1.77(s)	7.74(s)	6.18(t)	2.19(m,2H)	5.10(m)	3.95(m)	3.61(m)	2.97(m,2H); 1.29(m,4H); 0.85(t,3H)
5b	1.77(s)	7.73(s)	6.18(t)	2.18(m,2H)	5.12(m)	3.92(m)	3.61(m)	2.95(m,2H); 1.37(m,2H); 1.22(m,10H); 0.84(t,3H)
5c	2.12(s)	8.05(s)	6.47(t)	2.52(m,2H)	5.38(m)	4.22(m)	4.06(m)	3.51(m,1H); 1.95(m,5H);1.48(m,5H)
5d	1.76(s)	7.73(s)	6.17(t)	2.17(m,2H)	5.06(m)	3.92(m)	3.61(m)	
5e	1.77(s)	7.73(s)	6.17(t)	2.19(m,2H)	5.10(m)	3.91(m)	3.61(m)	2.96(m,2H); 1.36(m,6H);3.36(m,2H)
5f	8.37(s)	8.15(s)	6.34(dd)	2.93(m) 2.44(m)	5.28(d)	4.08(m)	3.65(m)	4.22(d,2H); 7.38(m,5H)
5g	8.37(s)	8.14(s)	6.35(dd)	2.91(m) 2.46(m)	5.23(d)	4.08(m)	3.61(m)	
5h	8.33(s)	8.12(s)	6.32(dd)	2.92(m) 2.40(m)	5.23(d)	4.06(m)	3.64(m)	2.97(m,2H); 1.48(m,6H);3.48(m,2H)

Product	Base ring		Sugar moiety					Carbamate moiety
	H6(H8)	H5(H2)	H1'	(H2')	H3'	H4'	(H5',H5'')	
7a	7.61(d)	5.58(d)	5.79(d)	4.16[b]	3.97[b]	3.97[b]	4.17[b]	4.18[b]; 7.26(m,5H)
7b	8.33(s)	8.14(s)	5.89(d)	4.66(t)	4.09[b]	4.10[b]	4.12(m,2H)	2.92(m,2H); 1.22(m,10H);0.83(t,3H)

[a] All samples were measured in DMSO-d_6 except **4e**, **4g** and **5c** (CD_3OD). [b] Superimposed signals.

Table 5. ^{1}H-NMR Data of Compounds **8a-c** and **9a-d** (non interchangable signals) δ(ppm).^{a}

Product	Base ring		Sugar moiety					Amino acid moiety	
	Me(H8)	H6(H2)	H1'	(H2',H2'')	H3'	H4'	(H5',H5'')	H_{α}	R
8a	1.80(s)	7.39(s)	6.22(t)	2.32(m)	4.49(m)	4.12(m)	4.32(m)	3.60(d,2H)	
8b	8.08(s)	7.89(s)	6.18(t)	2.65(m)	2.51(m)	4.58(m)	4.12^{b}	4.19^{b}	3.45(d,2H)
8c	8.10(s)	7.94(s)	6.20(t)	2.78^{b}	2.50(m)	4.56(m)	4.20^{b}	4.20^{b}	3.98(m,1H);2.70^{b}; 1.90(s,3H)
9a	1.90(s)	7.72(s)	6.35(t)	2.51(m)	5.23(m)	4.26(m)	3.92^{b}	3.97^{b}	
9b	2.05(s)	7.95(s)	6.52(t)	2.56(m)	5.45(m)	4.30(m)	4.00(m)	4.40(m,1H)	2.95-3.18(m,2H); 2.33(s,3H)
9c	2.08(s)	8.01(s)	6.51(t)	2.56(m)	5.42(m)	4.30(m)	4.02(m)	4.38(m,1H)^{b}	2.66(m,2H);2.41(m,1H) 2.15(m,1H) 4.35(q,2H)^{b}; 1.44(t,3H)
9d	8.15(s)	7.87(s)	6.22(dd)	2.70(m)	2.52(m)	5.21(m)	4.21(m)	3.72(m)	3.89(m,1H)2.40(m,2H); 1.92(m,1H);2.1(m,1H) 4.01(q,2H); 1.15(t,3H)

^{a} All samples measured in D_2O, except **9b** and **9c** (CD_3OD). ^{b} Superimposed signals.

Table 6.^{13}C-NMR Chemical shifts of Compounds **4a-g, 5a-h** and **7a-b** δ(ppm).^{a}

Product	Base ring					Sugar moiety					Carbamate moiety	
	C2	C4	C5	C6	Me(C8)	C1'	C2'	C3'	C4'	C5'	C=O	R
4a	150.74	163.97	109.93	136.22	12.43	84.07	38.95	71.00	84.55	64.32	156.22	40.52; 31.78; 19.66; 13.90
4b	150.49	163.69	109.69	135.91	12.14	83.81	38.62	70.73	84.22	64.36	156.24	43.80; 139.56; 128.26(2C) 127.06(2C); 126.84
4c	150.52	163.75	109.66	136.06	12.26	83.84	38.67	70.79	84.29	64.09	155.17	49.55; 32.74(2C); 25.17 24.68(2C)
4d	150.52	163.75	109.70	135.99	12.23	83.85	38.80	70.79	84.33	64.11	155.98	60.62; 40.53; 32.24; 29.37 22.84
4e	154.71	151.15	121.23	159.38	141.55	86.52	41.88	73.57	87.58	66.23	158.12	42.80; 33.85; 31.76; 28.74 31.26(2C); 24.58; 15.35
4f	152.75	149.30	119.50	156.50	139.40	83.22	38.74	71.11	84.84	64.09	156.43	
4g	154.75	15142	121.30	159.70	141.55	86.51	41.81	73.58	87.56	66.20	158.40	63.65; 42.65; 34.61; 31.52 24.99
5a	150.45	163.76	109.74	135.81	12.34	83.65	36.88	74.65	85.01	61.47	155.45	40.36; 31.49; 19.46; 13.70
5b	150.51	163.73	109.73	135.79	12.33	83.64	36.89	74.66	85.01	61.47	155.42	40.33; 31.27; 28.34; 26.28 28.70 (2C); 22.13; 13.99
5c	153.27	167.19	112.70	138.76	13.38	86.95	39.59	77.30	87.83	63.90	158.07	52.19; 34.96(2C); 27.47 27.02(2C)
5d	150.60	163.86	109.80	135.83	12.38	83.63	36.84	74.36	84.97	61.46	156.20	
5e	150.55	163.77	109.79	135.83	12.39	83.67	36.88	74.68	85.03	60.65^{b}	155.46	60.65^{b}; 40.14;32.22; 29.30 22.85
5f	152.53	148.96	119.29	156.22	139.5^{b}	84.10	37.01	75.39	85.74	61.91	155.83	43.8; 139.5^{b}; 128.38(2C) 127.10(2C); 126.91
5g	152.56	148.96	119.31	156.24	139.60	84.11	37.05	74.80	85.76	61.92	156.09	
5h	152.54	148.96	119.27	156.23	139.56	84.10	37.08	75.05	85.76	61.92	155.51	60.66; 40.38; 32.25; 29.34 22.88
7a	150.31	162.57	101.67	140.28	-	87.29	72.09	69.55	81.47	63.70	155.71	43.33; 139.17; 127.85(2C) 126.59(2C); 126.42
7b	152.95	149.84	119.22	156.2^{b}	139.69	87.10	73.21	70.86	82.65	64.29	156.18^{b}	41.20; 31.44; 29.62; 28.91 27.64; 26.50; 23.34; 14.22

^{a} All samples were measured in DMSO-d_6 except **4e**, **4g** and **5c** (CD_3OD). ^{b} Superimposed signals.

Table 7. ^{13}C-NMR Chemical shifts of Compounds **8a-c** and **9a-d** δ(ppm).[a]

Product	Base ring					Sugar moiety					Amino acid moiety			
	C2	C4	C5	C6	Me(C8)	C1'	C2'	C3'	C4'	C5'	C=O	CO_2^-	CH_α	R
8a	154.91	170.65	112.22	137.33	12.78	85.82	39.11	71.47	84.79	64.91	158.64	178.06	44.82	
8b	152.82	148.61	118.85	155.54	139.87	85.22	39.23	71.24	84.21	64.44	158.33	177.91	44.69	
8c	152.90	148.73	118.92	155.66	139.98	85.31	39.11	71.43	84.46	64.89	157.77	178.03	55.84	36.91; 15.27
9a	152.44	167.25	112.35	138.17	12.48	85.40	37.00	75.96	85.83	62.11	158.33	176.18	44.79	
9b	156.11	171.23	112.44	138.09	13.52	86.86	38.93[b]	77.02	87.04	63.45	157.98	178.02	57.21	38.93[b]; 16.43
9c	152.85	166.80	112.30	138.43	12.99	86.57	39.05	77.45	87.30	63.48	158.20	179.30	55.20	31.91; 28.41 62.17; 15.00 174.96
9d	152.63	148.43	119.25	155.77	140.81	86.33	37.71	76.48	85.72	62.43[b]	157.78	179.31	58.94	31.48; 25.94 62.43[b]; 13.92 176.40

[a] All samples measured in D_2O, except **9b** and **9c** (CD_3OD). [b] Superimposed signals.

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