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NUCLEOPEPTIDIC BIOCONJUGATES CONTAINING A SULFAMIDE BRIDGE: LINKAGE *VIA* THE *MITSUNOBU* REACTION

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Abstract The synthesis of compounds connecting unprotected 2'-deoxyribonucleosides (T,dC,dG,dA) with N-Boc sulfamoyl derivatives of natural aminoacid esters (Phe, Asp) was carried out by *Mitsunobu* reaction, using regiospecific coupling. The created link was *a priori* non-hydrolyzable in biological conditions.

Introduction

Chimeric nucleopeptides are potentially important compounds capable of interfering with crucial biological processes such as replication, transcription of nucleic acids and protein biosynthesis. In principle, a conjugated peptide moiety might enhance targeting of the nucleotide to an appropriate site. The linkage should be sufficiently stable to resist degradation by intra- and intercellular enzymes (esterases, peptidases, nucleases), and at the oligonucleotides level, the presence of the conjugated peptide at the 5'-end must not hinder hybridization with a complementary nucleic acid fragment. Several model systems have been investigated (1). We have previously described the chemical and enzymatic syntheses of nucleoside aminoesters which unite the 5'-terminal of the nucleoside to aminoacids by an ester link (2). These compounds however are potentially hydrolyzable under biological conditions. Accordingly, we have directed our attention to more stable linkages. We present here the synthesis of a new model of nucleopeptide bioconjugates which connects the 5'-nucleotide position with the amino terminal of aminoacids by means of a non-ionic sulfamoyl link; the nucleopeptidic interface thus defined is therefore of the type N↔5' (*Fig 1, left)* . This type of linkage should be highly resistant to biological cleavage.

On the other hand, the nucleotide phosphate group was replaced by a sulfamoyl group following the discovery of fungal antibiotics such as nucleocidine, 5'-sulfamoyladenosine or 5'-sulfamoyl-tubercidine(3). In addition, some structures contain both peptidyl and nucleosidyl moieties linked by a sulfamoyl bridge (i.e. *ascamycin, fig 1 right*). The proven therapeutic activity of these compounds is related to phosphate-sulfamate bioisosterism and the non-ionic character of analogs which allow the cellular membrane crossing.

Synthesis

The tBoc protected sulfamoylaminoesters **2-4** were synthesized utilizing the bifunctionality of chlorosulfonyl isocyanate (4) (scheme 1).The aminoacids used in the syntheses were the Me or tBu

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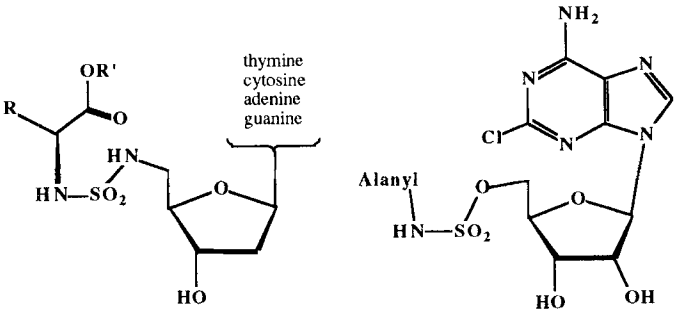
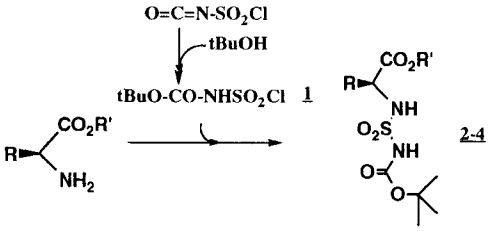


ascamycin

-Fig 1-



-scheme 1-

esters of L-phenylalanine (R=benzyl) and L-aspartic acid (R=CH_2COO*t*Bu). Synthesis of N-carboxylsulfamides **2-4** was conducted under the conditions previously described (5). Physicochemical and spectral characteristics are summarized in Table 1.

Coupling between the N-Boc-sulfamidaminoacid esters and unprotected natural deoxyribonucleosides (**T,dC,dA,dG**) was carried out by the Mitsunobu reaction (6) (scheme 2). The nucleophilicity of sulfonylcarbamic nitrogen in the peptidic moiety was enhanced by the cumulative electronic effects of both groups, thus leading to exclusive alkylation at that site. The chirality of aminoacid was not affected during this step.

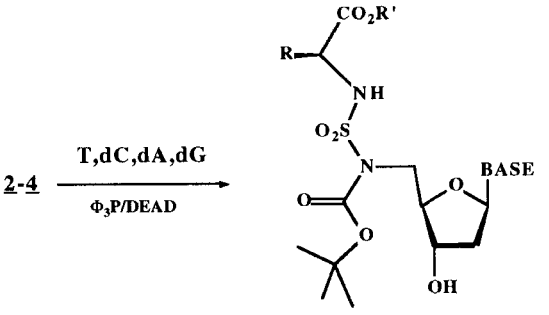
5'-Regiospecific substitution was achieved (total disappearance of the nucleoside) without side-product formation. Difficulties in eliminating triphenylphosphine oxide resulted in moderate yields of compounds **5-10**. Experimental conditions and spectroscopic data are summarized in Tables 2 and 3. To achieve efficient transformation the reactant had to be concentrated, and aprotic dipolar solvents were required.

In a prospective approach, the deprotection procedure was carried out starting from the conjugate **5** only. The N-tBoc group present on the sulfamoyl bridge could be easily eliminated by treatment with 25 % trifluoroacetic acid in dichloromethane (the methyl ester group constitutes an orthogonal protection of the aminoacid for the compound **11** derived from **5**. In contrast, the acidic treatment successively released the both protecting groups of conjugate **9** to ultimately provide **12** (scheme 3 and table 4).

TABLE 1 Selected physical and spectral data of sulfamoylaminoacid esters

Cpd	*R*	*R'*	*yield*	*Rf a*	*F°C*	*$[\alpha]_D$*	*NMR*	*MS*
2	Bn	Me	67	0.86	131	+12	1.5 (s, 9H, tBu); 3.1 (m, 2H, CH_2 Phe) 3.7 (s, 3H, CH_3O) 4.5 (m, 1H,CHα); 7.1 (s, 1H, NH Boc); 7.2 (m, 5H, Φ); 7.7 (d, 1H, NHCHα).	359 $(MH)^+$ 302, 259
3	Bn	tBu	72	0.88	108	+30	1.4 (s,9H,tBu); 1.5 (s,9H, tBoc); 3.1 (qd, 2H, CH_2Phe); 4.3 (m,1H, CHα); 7.05 (s,1H, NHBoc); 7.2 (m, 5H, Φ); 8.2 (d, 1H, NHCHα).	401 $(MH)^+$ 345, 289, 245
4	CH_2-CO_2tBu	tBu	69	0.83	---	- 8°	1.4 (m, 27H, 3xtBu); 2.6 (m, 2H, CH_2 Asp); 4.15 (m, 1H, CHα); 7.1 (s, 1H, NHBoc); 7.2 (m, 5H, Φ); 7.9 (d, 1H, NHCHα).	425 $(MH)^+$, 369, 313, 213

a dichloromethane:methanol 9:1; $[\alpha]_D^{25}$: (c=1; MeOH). MS (G-T matrix); FAB > 0; ^{1}H-NMR ($CDCl_3$)



5-10

-scheme 2-

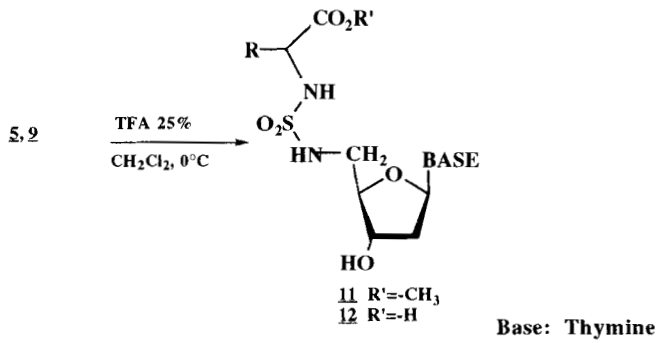
TABLE 2: Physical data of nucleosylsulfamoylaminoacid esters

Cpd	*Base*	*R*	*R'*	*solv*	*cond*	*react time (hrs)*	*yield (%)*	*Rf*	*F°C*	*$[\alpha]_D$* (e)	*molecular formula*	*elem.analysis calc* C,H,N,S	*found* C,H,N,S
5	T	Bn	Me	DMF	a	24	46	0.63 (c)	90-92	+72	$C_{25}H_{34}N_4O_{10}S$	51.59 5.88 9.62 5.51	51.62 6.07 9.54 5.23
6	C	Bn	Me	DMF	a	24	54	0.17 (c)	128-130	+ 74	$C_{24}H_{33}N_5O_9S$	50.83 5.86 12.35 5.65	51.12 6.14 12.47 5.33
7	G	Bn	Me	THF-HMPA	b	24	37	0.2 (d)	198 dec	+15.8	$C_{25}H_{33}N_7O_9S$	49.46 5.48 16.15 5.28	51.62 6.07 16.09 4.94
8	A	Bn	Me	THF-HMPA	b	24	32	0.4 (d)	162 dec	+ 9.2	$C_{25}H_{33}N_7O_8S$	50.76 5.58 16.58 5.41	50.58 5.48 16.41 5.14
9	T	Bn	tBu	DMF	a	24	47	0.5 (c)	78-80	+ 72	$C_{28}H_{40}N_4O_{10}S$	53.89 6.46 8.97 5.14	53.71 6.36 8.79 4.88
10	T	CH_2-CO_2tBu	tBu	DMF	a	24	27	0.5 (c)	40-42	+ 37	$C_{27}H_{44}N_4O_{12}S$	50.00 6.79 8.64 4.93	50.29 7.07 8.76 4.61

conditions: a: nucleoside=1eq; $P\Phi_3$=1eq; DEAD=1eq; carboxylsulfamide=1eq;
b: nucleoside=1eq; $P\Phi_3$=1.2eq; DEAD=1.2eq; carboxylsulfamide=1.2eq
(c) ethyl acetate, (d) dichloromethane:methanol 9:1; e: (c=1; MeOH).

TABLE 3 : NMR and MS Data of nucleosylsulfamoylaminoesters

Cpd	*^{1}H NMR (DMSO d6, 250 MHz)*	*MS FAB (NBA matrix)*
5	1.4 (s, 9H, tBu); 1.78 (s, 3H, CH_3-5); 2.2-2.4 (m, 2H, CH_2- 2'); 2.95 (qd, 2H, CH_2Phe); 3.55 (s, 3H, CH_3O); 3.73 (d, 2H, CH_2-5'); 3.9 (m, 1H, CH-4'); 4.15 (m, 1H, CH-3'); 4.3 (t, 1H, CHα); 5.35 (d, 1H, OH-3'); 6.15 (t, 1H, CH-1'); 7.2 (m, 5H, Φ); 7.5 (s, 1H, H-6); 8.4 (m, 1H, NHCHα); 11.3 (s, 1H, NH-3).	583 $(MH)^+$, 527
6	1.45 (s, 9H, tBu); 1.9-2.1 (m, 2H, CH_2–2'); 2.9 (qd, 2H, CH_2Phe); 3.6 (s, 3H, CH_3O); 3.7 (d, 2H, CH_2-5'); 3.9 (m, 1H, CH-4'); 4.1 (m, 1H, CH-3'); 4.3 (t, 1H, CHα); 5.35 (d, 1H, OH-3'); 5.76 (d, 1H, CH-5); 6.15 (t, 1H, CH-1'); 7.1-7.3 (m, 6H, =NH-4, Φ); 7.6 (d, 1H, CH-6); 8.5 (m, 1H, NHCHα); 11.3 (s, 1H, NH-3).	569 $(MH)^+$, 513, 469.
7	1.4 (s, 9H, tBu); 2.25 (m, 2H, CH_2-2'); 3.0 (m, 2H, CH_2Phe); 3.6 (s, 3H, CH_3O); 3.65 (d, 2H, CH_2-5'); 3.8 (m, 1H, CH-4'); 3.95 (m, 1H, CH-3'); 4.3 (t, 1H, CHα); 5.4 (d, 1H, OH-3'); 6.15 (t, 1H, CH-1'); 6.5 (s, 1H, NH_2-2); 7.2 (m, 5H, Φ); 7.85 (s, 1H, CH-8), 8.45 (m, 1H, NHCHα); 10.7 (s, 1H, NH-1).	608 $(MH)^+$, 552
8	1.4 (s, 9H, tBu); 2.3 (m, 1H, CH-2'); 2.9 (m, 3H, CH-2', CH_2Phe); 3.55 (s, 3H, CH_3O); 3.95 (m, 2H, CH_2-5'); 4.05 (m, 1H, CH-4'); 4.3 (q, 1H, CH-3'); 4.4 (m, 1H, CHα); 5.45 (d, 1H, OH-3'); 6.3 (t, 1H, CH-1'); 7.15-7.4 (m, 7H, Φ, NH_2-6); 8.14 (s, 1H, CH-8), 8.28 (s, 1H, CH-2); 8.5 (m, 1H, NHCHα).	592 $(MH)^+$, 536 .
9	1.4 (s, 9H, tBu); 1.5 (s, 9H, tBoc); 1.78 (s, 3H, CH_3-5); 2.15-2.35 (m, 2H, CH_2-2'); 3.1 (d, 2H, CH_2Phe); 3.9 (d, 2H, CH_2-5'); 4.1 (m, 1H, CH-4'); 4.25 (q, 1H, CH-3'); 4.35 (m, 1H, CHα); 5.35 (d, 1H, OH-3'); 6.3 (t, 1H, CH-1'); 7.25 (m, 5H, Φ); 7.4 (s, 1H, CH-6); 8.4 (m, 1H, NHCHα); 11.5 (s, 1H, NH-3).	625 $(MH)^+$, 569 .
10	1.4 (s, 27H, 3tBu); 1.8 (s,3H, CH_3-5); 2.15 (m, 2H, CH_2-2'); 2.5 (d, 2H, CH_2Asp); 3.8 (m, 2H, CH_2-5'); 4.0 (m, 1H, CH-4'); 4.15 (q, 1H, CH-3'); 4.2 (m, 1H, CHα); 5.25 (d, 1H, OH-3'); 6.15 (t, 1H, CH-1'); 7.5 (s, 1H, CH-6); 7.8 (d, 1H, NHCHα); 11.3 (s, 1H, NH-3).	647 $(M-H)^-$



-scheme 3-

TABLE 4. Selected physical and spectral data of deprotected nucleosylsulfamoylaminoacids

Product	*reaction time (hrs)*	*yield (%)*	*Rf*	*F°C*	*$[\alpha]_D$*	*^1H NMR (DMSO d_6, 250 MHz)*	*MS FAB (NBA matrix)*
11	4	71	0.38 (a)	68-70	+ 7	1.78 (s, 3H, CH_3-5); 2.0 (d, 2H, CH_2- 2'); 2.65 (m, 2H, CH_2-5'); 2.95 (qd, 2H, CH_2Phe); 3.5 (s, 3H, CH_3O); 3.7 (m, 1H, CH-4'); 3.9 (m, 1H, CHα); 4.05 (m, 1H, CH-3'); 5.3 (d, 1H, OH-3'); 6.1 (t, 1H, CH-1'); 7.1 (t, 1H, NH-5'); 7.2 (m, 5H, Φ); 7.45 (s, 1H, CH-6), 7.65 (d, 1H, NHCHα); 11.3 (s, 1H, NH-3).	483 $(MH)^+$.
12	4	77	0.25 (a)	120-125	+ 5	1.8 (s, 3H, CH_3-5); 1.98-2.09 (d, 2H, CH_2-2'); 2.6-2.7 (m, 2H, CH_2-5'); 2.8-3.1 (m, 2H, CH_2Phe); 3.68 (m, 1H, CH-4'); 3.9 (td 1H, CHα); 4.05 (m, 1H, CH-3'); 5.1-5.3 (m, 1H, OH-3'); 6.1 (t, 1H, CH-1'); 7.08 (t, 1H, NH-5'); 7.15-7.3 (m, 5H, Φ); 7.45 (s, 1H, CH-6); 7.38 (d, 1H, NHCHα); 11.3 (s, 1H, NH-3); 12.7 (s, 1H, CO_2H).	469 $(MH)^+$

(a) ethyl acetate, $[\alpha]_D^{25}$:(c=1; MeOH).

According to the NMR data, the carboxylsulfamides show two easily identifiable exchangeable NH's signals (d and s). After condensation, the singlet signal disappeared, the 5'-substituted osidic methylene and the 4'-proton signals deshielded by 0.30 to 0.45 ppm as compared to the initial nucleoside. This clearly establishes the specificity of the nucleophilic substitution with the initial synthons (carbamic NH and 5'-CH_2OH).

The elimination of the Boc group at the sulfamoyl bridge can be seen by a significant shielding of the $C^5'H_2$ (∂=2,55-2,75ppm) and the appareance of a triplet at 7.15 ppm corresponding to the C^5'-NH proton. No intramolecular hydrogen bonding between the peptidic and nucleic moieties was revealed by 2D-NMR COSY and NOESY for "Phe-sulfamoyl-Thymidine" with and without the protecting groups (respectively **9** and **12**).

All the chimerical nucleopeptidic compounds shown here were subjected to evaluation of antiviral activity* (towards 12 DNA/RNA viruses, including HIV). Cytotoxic concentrations varied between 10 and 100 µM depending on the cell strains affected. There was no significant antiviral protection observed in the weakest concentrations tested.

Conclusion

Despite these results, this type of linkage can be considered as an elementary interface of larger nucleopeptidic hybrids, which could be prepared with the goal of modified nucleoside (or oligonucleotide) vectorisation by peptides. The type of coupling that we have developed is logically compatible with repetitive chemical syntheses of nucleic and peptidic oligomers, since it connects the N-terminal aminoacid of a peptide and the 5'-terminal nucleoside of an oligonucleotide. Coupling conditions appear to be appropriate for the manipulation of unprotected nucleosides.

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Experimental section

Melting points were determined in open capillary tubes on a Thermotechnal apparatus and are uncorrected. Microanalysis were performed in the microanalysis laboratory of ENSCM (Montpellier). Proton and ^{13}C nuclear magnetic resonance were determined with a AC 250 Bruker spectrometer. Fast-atom bombardment mass spectra (FAB-MS) were recorded in the positive or negative ion mode on a JEOL DX 300 mass spectrometer and the matrix was glycerol (G), a mixture of glycerol and thioglycerol (50/50 v/v) (GT) or metanitrobenzyl alcohol (NBA). Optical rotations were mesured in a 1 cm cell on a Perkin-Elmer Model 241 spectropolarimeter. Thin layer chromatography (TLC) was performed on precoated aluminium sheets of silica gel 60 F_{254}.

General procedure for the sulfamoylation of aminoesters

Tert-butanol (5,75 mL; 61,3 mmole) in an isobar funnel was slowly added to a solution of chlorosulfonyl isocyanate (5,34 mL; 61,3 mmole) in dry dichloromethane(25 mL). The solution was stirred 15 mn and the resulting chlorosulfonylcarbamate added dropwise to a solution of aminoester (55,8 mmole) and triethylamine (67 mmole) at 0°C in dichloromethane. After about 4 hrs stirring the reaction was completed (tlc: $Rf_{sulfamide}$ = 0,86. CH_2Cl_2-MeOH: 9/1, ninhydrine). The mixture was diluted with dichloromethane, washed with a solution of 3% citric acid (3x50mL), and then with water (3x50mL). The organic layer was dried with sodium sulphate and evaporated in vacuo. The resulting oil was purified by chromatography on a silica gel column (eluent: ethyl acetate).

General procedure for Mitsunobu reaction

A solution of diethylazodicarboxylate (0,54 g; 3,1 mmole) and carboxylsulfamide (3,1 mmole) in 2 mL dry solvent (table 2) was slowly added to a solution of nucleoside (2,06 mmole) and triphenylphosphine (0,813 g; 3,1 mmole) in the same solvent (2 mL) (rt). The solution was stirred overnight. Tlc (AcOEt) indicates the formation of the expected compound, revealed by UV lamp, sulphuric acid (20% in water) and ninhydrin. The solvent was evaporated in vacuo and the resulting residue purified by chromatography on a column of silica gel (eluent:methanol gradient in dichloromethane from 0 to 10%). The product obtained was recrystallized in ether-hexane.

General procedure for deprotection

A solution of trifluoroacetic acid (2mL) in 2 mL of anhydrous dichloromethane was slowly added to a solution of nucleosylaminoester **5**-**10** (0,1 g) in 4 mL of dry dichloromethane at 0°C. Tlc analysis indicates after 4 h of reaction the formation of a compound less polar than the precursor. The solvent was evaporated in vacuo and the residue triturated several times with ether, giving a crude solid. A white powder was obtained after crystallization.

References

1. **a.** Juodka BA, *Nucleosides Nucleotides* (1984) **3**, 445. **b.** Robles J, Pedroso E, Grandas A. *Tetrahedron Lett* (1991) **32**, 4389. **c.** Schattenkerk C, Wreesman CTJ, de Graaf MJ, van der Marel GA, van Boom JH, *Tetrahedron Lett* (1984) **25**, 5197. **d.**Kuyl-Yeheskiely E, van der Klein PAM, Visser GM, van der Marel GA, van Boom JH, *Recl Trav Chim Pays-Bas* (1986) **105**, 69. **e.**Kuyl-Yeheskiely E, Tromp CM, Schaeffer AH, van der Marel GA, van Boom JH, *Nucleic Acids Res* (1987) **15**, 1807. **f.** Kuyl-Yeheskiely E, Tromp CM, Lefeber AWM, van der Marel GA, van Boom JH, *Tetrahedron* (1988) **44**, 6515. **g.**Kuyl-Yeheskiely E, Dreef-Tromp CM, Geluk A, van der Marel GA, van Boom JH, *Nucleic Acids Res* (1989) **17**, 2897. **h.** Dreef-Tromp CM, van Dam E, van den Elst E, van der Marel GA, van Boom JH, *Nucl Acids Res* (1990) **18**, 6491. **i.** Hotoda H, Ueno Y, Sekine M, Hata T, *Tetrahedron Lett* (1989) **30**, 2117.
2. **a.** Montero J-L, Criton M, Dewynter G-F, Imbach J-L. *Tetrahedron Lett* (1991) **32**, 5357. **b.** Criton M, Dewynter G-F, Montero J-L, *Recl Trav Chim Pays-Bas* (1991) **110**, 433.

3. **a.** Suhaldonik RJ, *Prog Nucleic Acid Res Mol Biol* (1979) **22**, 193. **b.** Castro-Pichel J, Garcia-Lopez MT, Herranz R, Perez C, *Nucleosides Nucleotides* (1990) **9**, 985.
4. **a.** Graf R, *Angew Chem* (1968) **80**, 179. **b.**Graf R, *Angew Chem Int Ed Engl* (1968) **7**, 172. **c.**Rasmussen JK, Hassner A, *Chem Rev* (1976) **76**, 389. **d.** Szabo WA, *Aldrichimica Acta* (1977) **10**, 23. **e.** Dhar DN, Murthy KSK, *Synthesis* (1986) 437.
5. **a.** Montero J-L, Dewynter G-F, Agoh B, Delaunay B, Imbach J-L,*Tetrahedron Lett* (1983); **24**; 3091. **b.** Agoh B, Dewynter G-F, Montero J-L, Leydet A, Imbach J-L, *Bull Soc Chim Fr* (1987 867.
6. **a.** Mitsunobu O, *Synthesis* (1981) 1. **b.**Kim M-J, Hennen WJ, Sweers HM, Wong C-H, *J Am Chem Soc* (1988) **110**, 6481. **c.** Wada M, Mitsunobu O, *Tetrahedron Lett* (1972) 1279. **d.** Koppel I, Koppel J, Degerbeck F, Grehn L, Ragnarsson U, *J Org Chem* (1991) **56,** 7172.

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