NOTE

A ONE POT TRITIATION OF AMINOGLYCOSIDE ANTIBIOTICS

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A rapid and efficient method of labeling molecules is always welcome. The introduction of ^{3}H or ^{14}C in aminoglycoside antibiotics seems to be difficult because these molecules are polyfunctional[1]. This structural feature needs of course protective and deprotective steps; this makes the access of these molecules rather difficult. We wish to report here a one pot synthesis of tritiated aminoglycoside antibiotics (Fig. 1) which was necessary to study their biological properties, especially their binding to receptor sites and their transport through bacterial membranes.

It is well known that primary and secondary amino functions can be oxidized to the corresponding imines with various reagents[2,3]. Sodium hypochlorite is one of them and appeared to us to be appropriate for our purpose: it does not react with primary or secondary hydroxyl functions under standard conditions[4]. The reduction of imino groups by a borohydride is also well documented in the literature and leads to the corresponding amino group[5]. Based on these observations, we decided to study the behavior of aminoglycoside antibiotics submitted sequentially to sodium hypochlorite and tritiated borohydride as shown in Fig. 2.

Fig. 2. Reactions leading to the tritiation of the aminoglycoside antibiotic.

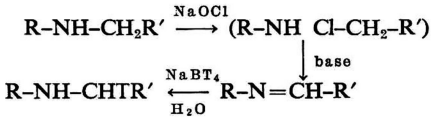
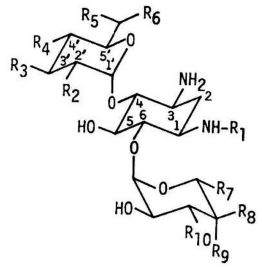


Fig. 1. Structure of the aminoglycoside antibiotics submitted to the novel tritiation method.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Tobramycin (**1a**)	H	NH_2	H	OH	H	NH_2	CH_2OH	OH	H	NH_2
Amikacin (**1b**)	CO–CHOH–$(CH_2)_2NH_2$	OH	OH	OH	H	NH_2	CH_2OH	OH	H	NH_2
Kanamycin A (**1c**)	H	OH	OH	OH	H	NH_2	CH_2OH	OH	H	NH_2
Gentamicin C_{1a} (**1d**)	H	NH_2	H	H	H	NH_2	H	CH_3	OH	$NHCH_3$
Gentamicin C_1 (**1e**)	H	NH_2	H	H	CH_3	$NHCH_3$	H	CH_3	OH	$NHCH_3$
Gentamicin C_2 (**1f**)	H	NH_2	H	H	CH_3	NH_2	H	CH_3	OH	$NHCH_3$
Sisomicin (**1g**)	$\Delta^{4',5'}$-Gentamicin C_{1a}									
Netilmicin (**1h**)	N^1-Ethylsisomicin									

Table 1. Experimental conditions and results obtained in the tritiation of representative aminoglycoside antibiotics.

Aminoglycoside	Base used	ClONa / Aminoglycoside	Reaction time (minutes)	Temperature (°C)	$NaBT_4$	Yield after chromatography (%)	% of tritium incorporated	Specific activity (Ci/mole)
	$NaHCO_3$ + resin OH^-	0.9	11	0	1.13×10^{-5} mole[a] A/H=1/6	82	15	0.74
Gentamicin C_2 (1f)	$NaHCO_3$	0.75	30	0	1×10^{-4} mole[a] A/H=2/1	74.2	1.86	9.95
	$NaHCO_3$	0.75	3	0	1×10^{-5} mole[b] A/H=2/1	50	0.31	50
	KOH	1.1	2	−10	0.167×10^{-5} mole[c] A/H=1	33	1.4	162.5
Netilmicin (1h)	$NaHCO_3$	0.9	5	0	2.9×10^{-5} mole[a] A/H=1/2	97	1.5	0.24
	$NaHCO_3$	1.1	5	−11	0.39×10^{-5} mole[a] A/H=1/16	98	8.6	0.17
Sisomicin (1g)	$NaHCO_3$	0.9	10	0	1.05×10^{-5} mole[a] A/H=1/6	85.8	1.7	0.7
Amikacin (1b)	$NaHCO_3$ + resin OH^-	1.1	20	−10	6.25×10^{-5} mole[a] A/H=1	65.6	0.52	0.19
	KOH	1.1	8	−10	6.25×10^{-5} mole[a] A/H=1	61.4	2.6	1.05
Kanamycin A (1c)	$NaHCO_3$ + resin OH^-	1	25	0	1.32×10^{-5} mole[a] A/H=1/4	91.8	1.5	0.11

[a] 100 Ci/mole; [b] 10 Ci/mole; [c] 15 Ci/mole.

A typical example of the method is described below. An 0.9 equivalent of sodium hypochlorite was added to a solution of gentamicin C_2 (139 mg, 0.3×10^{-3} mole) in 2 ml of 0.14 M sodium bicarbonate cooled at 0°C. After 1 minute, the solution was made alkaline by adding 10 mg of Amberlite IR-4B resin (OH^- form). The solution was stirred for 10 minutes at 0°C before removing the resin by filtration. An aliquot of tritiated sodium borohydride (100 Ci/mole) was then added to the solution, which was again stirred for 5 minutes before adding 10 mg of sodium borohydride to complete the reaction Stirring was continued for 90 minutes at 0°C prior to lyophilization, and the residue was submitted to a first chromatography on silica gel (Merck 230~400 mesh) using as eluent a mixture of methanol - ammonia - water - chloroform (3: 1.5: 0.5: 1). The crude gentamicin C_2, thus isolated, was placed on a column of Amberlite CG-50 (NH_4^+ form, pH 6.8), which was thoroughly washed with distilled water. Elution of the antibiotic was performed with 0.4 M NH_4OH. After lyophilization 114 mg (82%) of pure gentamicin C_2 was recovered with a specific radioactivity of 0.75 Ci/mole, which represents an incorporation of 15% of the tritium. The radioactivity was located on the garosamine moiety, as shown by splitting gentamicin according to the method of MEYER ZU RECKENDORF and BISCHOF[6]. This result is in agreement with the fact that secondary amino functions are more susceptible than primary amino groups to the reagent used here.

The results are summarized in Table 1. The purity of every labeled antibiotic has of course been checked by TLC. For some antibiotics, the experimental conditions were modified slightly: such modifications are also reported in Table 1. The efficiency of the procedure depends essentially on three factors. The base used appears to be important. Amikacin, for instance, is poorly labeled when sodium bicarbonate is used, whereas it is well labeled in the presence of KOH. It has been reported[7] that KOH is a better reagent than NaOH and, of course, sodium bicarbonate, for dehydrohalogenation reactions. The temperature is also critical, as shown by the tritiation of netilmicin. A low temperature (−11°C) favors tritium incorporation. This can probably be explained by a stability associated with a reasonable reactivity of the imino function with the borohydride in the reaction medium. The ratio, sodium borohydride/aminoglycoside, is in fact the most prominent factor in the present procedure, as an excess of reducing agent leads to a more rapid reaction with non tritiated molecules due to an unfavorable isotopic effect (H^- ions react more quickly than T^- ions).

In conclusion, due to its simplicity and its efficiency, the present procedure appears to be a pertinent method of incorporating tritium into aminoglycoside antibiotics. It can be applied to almost all molecules not susceptible to the action of sodium hypochloride, and possessing either primary or secondary amino functions.

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