Biologically active acyclonucleoside analogues. II. The synthesis of 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine (BIOLF-62)

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The chemical synthesis of 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine is described. This compound, known as BIOLF-62, is active against herpesviruses. This compound is a member of a novel class of nucleoside analogues which lack a rigid carbohydrate ring, but which possess all of the functional groups of naturally occurring deoxynucleosides.

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L'auteur décrit la synthèse chimique du 9-[[2-hydroxy-1 (hydroxyméthyle)éthoxy]méthyl]guanine. Ce composé, connu comme le BIOLF-62, est actif contre les virus de l'herpès. Ce composé fait partie d'une nouvelle classe d'analogues nucléosides à qui il manque un anneau rigide d'hydrates de carbone, mais qui possèdent tous les groupes fonctionnels des déoxynucléosides formés naturellement.

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The search for biologically active molecules has focused a great deal of attention on modified nucleosides (1–3). For several years we have been developing a nucleoside analogue structure intended to interfere with viral and bacterial systems. The structure was designed to lack the rigid conformational feature (*syn–anti*) of natural nucleosides but at the same time retain the essential chemical features of natural nucleosides (e.g., purine or pyrimidine ring, $O^{3'}$, $O^{4'}$, and $O^{5'}$ oxygen functionalities). In a preliminary report (4) we described the synthesis of the adenine analogue 9[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]adenine. This compound was neither a substrate nor an inhibitor for adenosine deaminase, a key enzyme in animal systems that inactivates in vivo most adenine derivatives that show promising biological activity in vitro. In this report we wish to describe the synthesis of another member of this series, the potent antiviral compound 9[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine (**5**) which is known as BIOLF-62.

Results and discussion

The first route fully explored for the synthesis of BIOLF-62 involved the condensation of 6-chloroguanine[2] (**2**) with 1,3-dibenzyloxy-2-chloromethoxypropane (**1**, ref. 4). The procedure was based on the mercuric cyanide – silylated base procedure (5). Thus, 6-chloroguanine was silylated with HMDS and coupled with **1** in refluxing benzene using $Hg(CN)_2$ and ammonium sulfate as catalysts. The condensation was generally complete within 3 h. Yields were in the 80% range for both small and large scale reactions.

Tetrabutylammonium iodide has been proposed (6) as a mild catalyst in coupling reactions of this type. Thus when **1** and **2** were condensed in acetonitrile using a catalytic amount of $(n\text{Bu})_4\text{NI}$ a fairly rapid reaction occurred. The desired product **3** was isolated in 38% yield. Finally, the Hilbert–Johnson coupling method (as elaborated by Niedballa and Vorbuggen (7)) using stannic chloride gave very low yields of desired product.

The 6-chloroguanine derivative **3** was converted into the desired guanine derivative **4** by a standard procedure (5). These conditions employ sodium methoxide, mercaptoethanol, and a trace of water in methanol at reflux for 2 to 3 h. After work-up the product **4** was obtained in 70% yield.

The removal of the benzyl groups was easily accomplished by either of two routes. In the first

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[2]Proper name is 2-amino-6-chloropurine but is sold commercially as 6-chloroguanine.

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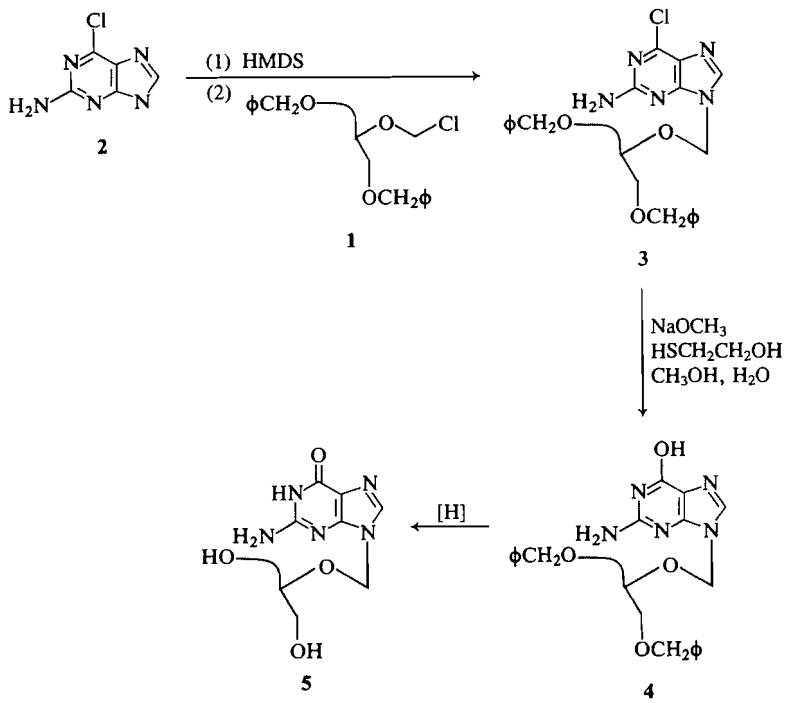
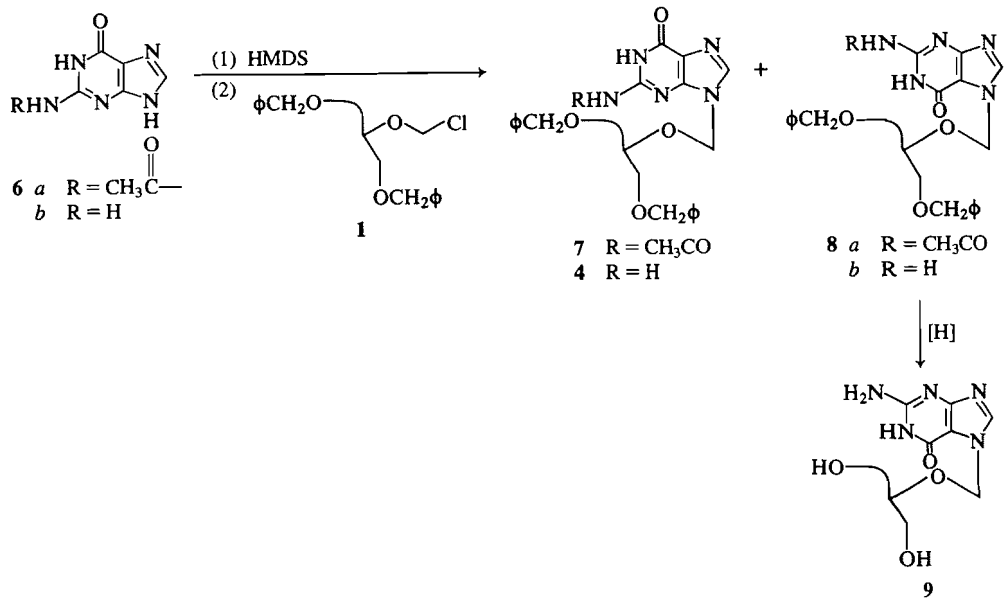


2 1 3 4 5

case, catalytic transfer reduction (8) using palladium black and cyclohexene in refluxing ethanol gave an 84% yield of **5** after 18 h. The alternate procedure used was a Birch reduction which produced **5** in similar yields. The Birch reduction has the advantage of being a faster procedure (~ 15 min).

The synthesis of **5** from 6-chloroguanine proved to be straightforward and easy to carry out. However, it suffers from the fact that 6-chloroguanine is very expensive and sometimes difficult to obtain. Alternate routes to **5** were sought and these included the condensation of *N*-acetylguanine with **1**. Several standard condensation procedures were investigated including the use of TBAI, molecular sieves, stannic chloride, and mercuric cyanide as condensation catalysts. The TBAI reaction gave the best results with 28% yields each for the N-9 isomer **7** and the N-7 isomer **8***a*.

In another experiment, guanine was condensed



6 *a* R = CH_3C—
b R = H

1

7 R = CH_3CO
4 R = H

8 *a* R = CH_3CO
b R = H

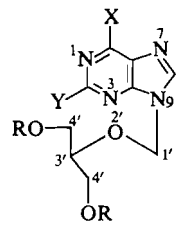
9

TABLE 1. Physical properties of guanine derivatives

Compound	Melting point (°C)	R_f*	λ max† pH 1	λ max† pH 7	λ max† pH 13
3	70–75	0.25(a)	246, 312	246, 310	246, 309
4	181–183	0.40(b)	256, 275(s)	252, 270(s)	258, 266
5	> 285 (dec.)	0.42(c)	255, 275(s)	253, 270(s)	258, 266
7	143–145	0.37(d)	262	258, 281(s)	263
8*a*	133–135	0.55(d)	264	264, 274(s)	268
8*b*	235–238	0.40(b)	250, 273	248, 282	279
9	> 290 (dec.)	0.44(c)	252, 270(s)	243, 287	282
7-Methylguanine			250, 271(s)	248, 285	280
N^2-methylguanine			258, 275	256, 270	253, 268
Guanosine			256, 273(s)	252, 270(s)	259, 266

*Thin-layer chromatography, Merck analytical sheets #5735. Solvents used were: (a) EtOAc–hexane (3:1); (b) EtOAC–MeOH (3:1); (c) iPrOH–NH_4OH–H_2O (7:1:2); (d) $CHCl_3$–MeOH (9:1).
†Samples dissolved in methanol–water (1:1) except for 5 where water was the solvent.

TABLE 2. ^{13}C nuclear magnetic resonance data* (δ ppm)



3, X = Cl, Y = NH_2, R—$CH_2\phi$
4, X = OH, Y = NH_2, R = $CH_2\phi$
5, X = OH, Y = NH_2, R = H

Compound	C-2	C-4	C-5	C-6	C-8	C-1′	C-3′	C-4′	—$\overset{*}{C}H_2\phi$
3	154.19	149.55	123.33	160.01	143.24	70.19	77.04	69.65	72.19
4	153.96	151.43	116.69	156.93	138.37	71.69	76.60	69.75	72.29
5	153.96	151.43	116.52	157.04	137.89	71.64	80.16	60.96	

*^{13}C Spectra were recorded in DMSO-d_6 on a Bruker WP90 spectrometer.

directly to **1** using TBAI as catalyst. The slow step in this route was the silylation of guanine which required 4 days. The condensation proceeded smoothly giving a high yield in the initial condensation. However, the product was largely a 7:3 mixture of **4** and **8***b* which had similar chromatographic properties in all solvents investigated. Fortunately it was found that a mixture containing only **4** and **8***b* could be separated by fractional crystallization from ethanol. The N-7 isomer **8***b* precipitates fairly rapidly and can be separated. The N-9 isomer **4** begins to crystallize after the N-7 isomer has been removed. In this way a 41% overall yield of **4** was obtained.

The N-9 isomers were fairly easy to characterize by direct (5) comparison of their properties to known N-9 alkylated guanines including the natural nucleoside guanosine. The assignment of the N-7 isomers is also based on the spectroscopic properties. The physical properties of all compounds are collected in Tables 1 to 4.

Sensitivity of various viruses to BIOLF-62

Eight different herpesviruses from six animal species were tested for their susceptibility to BIOLF-62. Four of these viruses, equine herpesviruses 1 and 3 and human herpesviruses 1 and 2, were extremely sensitive to the drug, all being inhibited by 50% (effective dose, 50%) at concentrations between 0.03 and 0.3 μg/mL. Swine and bovine herpesviruses (pseudorabies and infectious bovine rhinotracheitis virus) were slightly sensitive, being 50% inhibited at 5–6 μg/mL. Feline and canine herpesviruses were insensitive, requiring 32 or more μg/mL for inhibition. Several other viruses were tested and were found resistant (human cytomegaloviruses, herpes zoster virus, vaccinia virus, and human adenovirus (9).

TABLE 3. Proton magnetic resonance data for guanine derivatives* (δ ppm)

Compound	H-8	NH_2	H-1′	H-3′	H-4′	$—CH_2\phi$	Aromatic
3	8.24		5.54	4.04	3.38	4.34	7.20
4	7.80	6.50	5.46	4.02	3.38	4.20	7.24
5	7.80	6.50	5.44	3.40 (m, 5H)			
7	8.10		5.56	4.00	3.38	4.40	7.20
8*a*	8.36		5.74	4.10	3.34	4.36	7.24
8*b*	8.10	6.20	5.68	4.10	3.34	4.40	7.24
9	8.13	6.20	5.72	3.37			

*Spectra were recorded in DMSO-d_6 (TMS as internal standard) on a Varian XL-200 spectrometer. Chemical shifts are reported in ppm downfield from TMS. The NH_2 protons were exchangeable with D_2O. For compounds **5** and **7** the OH protons appeared at δ = 4.60. For compound **7** the acetyl protons appeared at δ = 2.14. The numbering system is as described in Table 2.

TABLE 4. Elemental analyses

Compound	Formula	Calculated (%)			Found (%)		
		C	H	N	C	H	N
3	$C_{23}H_{24}N_5O_3Cl$	60.86	5.33	15.43	60.91	5.37	15.32
4	$C_{23}H_{25}N_5O_4$	63.43	5.79	16.08	63.43	5.75	16.14
5	$C_9H_{13}N_5O_4 \cdot H_2O$	39.56	5.53	25.63	39.41	5.30	25.69

Experimental

*Preparation of 9-[[2-benzyloxy-1-(benzyloxymethyl)ethoxy]-methyl]-6-chloroguanine (**3**)*

Method A

6-Chloroguanine (15 g, 0.088 mol) was suspended in 1,1,1,3,-3,3-hexamethyldisilizane(HMDS, 200 mL). Ammonium sulfate (1.5 g) was added and the mixture was heated at reflux with exclusion of moisture for 2 h. The, by now clear, solution was concentrated at reduced pressure to leave a pale yellow solid. Mercuric cyanide (24 g, 0.095 mol) and benzene (250 mL) were added and the system was heated to reflux. To the refluxing solution was added compound **1** (29.93 g, 0.093 mol) dissolved in benzene (250 mL). The system was heated at reflux under a nitrogen atmosphere for a period of 3 h. The benzene was removed at reduced pressure and the residue was stirred with 1.5 L of dichloromethane. The solution was collected by filtration and washed twice with a 30% aqueous solution of potassium iodide (300 mL), twice with 10% aqueous potassium carbonate solution (300 mL), twice with water (300 mL), and finally with saturated sodium chloride solution (300 mL). The solution was then dried over sodium sulfate and evaporated at reduced pressure to leave 55 g of crude product.

The crude product was dissolved in a minimum volume of dichloromethane and applied to a silica gel column (8.5 × 30 cm) prepared in EtOAc–hexane (2:3). The column was eluted first with 2L of EtOAc–hexane (2:3) followed by 3 L of EtOAc–hexane (75:25). The desired product was eluted by the more polar solvent. A total of 32 g (80%) of compound **3** was obtained in this manner. Physical properties are collected in Tables 1–4.

*Conversion of **3** into 9-[[2-benzyloxy-1-(benzyloxymethyl)-ethoxy]methyl]guanine (**4**)*

Compound **3** (24 g, 0.05 mol) was dissolved in dry methanol (500 mL) and an additional 200 mL of methanol containing 4.6 g of sodium were added, followed by mercaptoethanol (16 mL) and water (1 mL). The resulting solution was refluxed under nitrogen for 1.5 h. At this point an additional 3 g of sodium in 50 mL of methanol was added and refluxing was continued for 1 h. The solution was concentrated at reduced pressure to approximately 70 mL and poured into water (400 mL). The pH of the solution was adjusted to pH 6 with glacial acetic acid. A yellow precipitate, which was collected by filtration and washed thoroughly with water and then ether, formed immediately. The solid was dried under vacuum (22 g). The nmr spectrum of the crude product indicated the presence of a major and minor (~ 20%) component. The solid was therefore stirred with warm ethyl acetate, collected by filtration, and crystallized from absolute ethanol to give 16 g (70%) of pure compound **4**. Physical properties are collected in Tables 1 to 4.

Method B

6-Chloroguanine (1.8 g, 10.6 mmol) was heated at reflux in HMDS (40 mL) containing ammonium sulfate (200 mg) until it became clear (2 h). The solvents were removed at reduced pressure and the residue was dissolved in dry acetonitrile (60 mL). To this solution was added tetra-*n*-butyl ammonium iodide (TBAI, 40 mg) followed by compound **1** (11 mmol) dissolved in acetonitrile (40 mL). The resulting solution was heated at reflux under a nitrogen atmosphere for 2 h. The solvents were then removed at reduced pressure and the residue was dissolved in methanol (100 mL) and the solution was stirred for 2 h. Again solvents were removed and the residue was dissolved in dichloromethane (300 mL) and the solution was filtered through Celite. After removal of solvents, 5.5 g of a viscous liquid was obtained. Pure **3** was isolated from the above liquid by flash chromatography on silica gel (Merck silica gel 60, 5 × 15 cm). The residue was dissolved in the minimum amount of dichloromethane and applied to the column which was eluted with hexane – ethyl acetate (1:3, 1 liter). Fractions of 50 mL were collected and fractions #13–19 contained the desired compound **3** (1.83 g, 38%).

Condensation of N*-acetylguanine (**6**a) and **1***

Method A

N-Acetylguanine (1.08 g, 5.65 mmol) was heated at reflux for 16 h in HMDS (50 mL) containing ammonium sulfate (50 mg). Solvents were removed at reduced pressure and the residue was dissolved in acetonitrile (75 mL) along with compound **1** (6 mmol) and TBAI (50 mg) and the solution was heated at reflux overnight. A mixture of methanol (40 mL) and water (10 mL)

was added and the solution was stirred for 1 h. Solvents were removed at reduced pressure and the products were isolated by silica gel column chromatography (20 × 5.5 cm) using methanol – ethyl acetate (7:93) as eluant. Fractions of 20 mL were collected every 20 min. Fractions 10 to 30 were combined and gave 1 g of a mixture of compounds that were further separated on silica thick-layer plates developed in 5% methanol in dichloromethane. Three bands were obtained containing 490 mg, 204 mg, and 250 mg, respectively. The latter material was the 7-alkylated isomer (**8***a*) and the others were unidentified. Fractions 30 to 50 were combined to give 500 mg of the 7-alkylated derivative (**8***a*) for a total yield of 750 mg (28%, Tables 1 and 3). The ^{13}C spectra showed important peaks at δ 173.23 (C=O), 23.53 (CH_3), 74.78 (C-1'), 76.45 (C-3'), 69.60 (C-4'), and 72.08 (—$\overset{*}{C}H_2\phi$)). Fractions 52 to 85 were combined to give 750 mg of the desired N-9 product **7** (28%, Tables 1 and 3). The ^{13}C spectra showed important peaks at δ 173.34 (C=O), 23.69 (CH_3), 72.19 (C-1'), 76.83 (C-3'), 69.65 (C-4'), and 72.19 (—$\overset{*}{C}H_2\phi$).

Method B

This procedure was similar to method A, except that the condensation between **1** and **6***a* was carried out in dichloroethane (75 mL) at reflux for 4 h over molecular sieves (4 Å). On work-up the product distribution was found to be virtually the same as that for method A.

Method C

This procedure was similar to method B, except that freshly distilled stannic chloride (1 mL) was used as catalyst and the reaction (in dichloroethane) was allowed to stand overnight at room temperature. The solution was poured into aqueous sodium bicarbonate solution and the products were extracted into chloroform. On work-up the product distribution was found to be 0.15 g (3%) of the 7-isomer (**8***a*), and 0.43 g (9%) of the 9-isomer (**7**).

Method D

This procedure was similar to method C, except that the solvent was benzene (90 mL) and the catalyst was mercuric cyanide. After refluxing for 1½ h the products were isolated as before to give 1.03 g of the 7-isomer (**8***a*, 22%), and 0.68 g (14%) of the 9-isomer (**7**).

Conversion of **7** *into* **4**

Compound **7** (1.3 g, 2.7 mmol) was dissolved in pyridine (1.5 mL) and concentrated ammonium hydroxide (6 mL) was added. The solution was heated at 55°C in a tightly stoppered flask for 15 h. The product **4** crystallized from solution as it was produced and 1.18 g (99%) was collected in this manner.

Conversion of **8**a *into* **8**b

This procedure was identical to that described above for **7** and the product **8***b* was obtained in 95% yield.

Conversion of **4** *into 9[[2-hydroxy-1-(hydroxymethyl)ethoxy]-methyl]guanine* **5**

Method A. Catalytic transfer hydrogenation

Compound **4** (15.6 g, 36 mmol) was dissolved in refluxing ethanol (800 mL). Cyclohexene (400 mL) and palladium black (15 g) were added and refluxing was continued for 18 h. The solution was collected by filtration and the palladium catalyst was extracted five times with hot DMF (200 mL, 100°C). The filtrate and washings were combined and evaporated to leave a residue which was dissolved in hot ethanol–water (200 mL each) and filtered through Celite containing 0.5 g of activated charcoal. The solvent was removed at reduced pressure and the residue crystallized from ethanol–water (4:1). A total of 7.72 g (84%) of **5** was recovered.

Method B. Birch reduction

A two-liter three-necked flask fitted with a Dry Ice condenser was cooled in an acetone – Dry Ice bath under a stream of nitrogen. About one liter of liquid ammonia was collected in the flask by condensing dry ammonia gas. A slurry of the compound **4** (20 g, 45.7 mmol) in dry THF (100 mL) was added with stirring. The temperature of the bath was then allowed to rise to −45° to −40°C. Sodium was then added in small quantities until a permanent blue colour was obtained. The stirring was continued for 15 min. The blue colour was then discharged by the addition of ammonium chloride in small quantities. (Note: All the above operations were carried out under nitrogen.) The ammonia and THF were then evaporated off under a stream of nitrogen. The white residue obtained was agitated with benzene (300 mL) and filtered. The solid left behind was dissolved in water (500 mL) and extracted with benzene (3 × 200 mL). The aqueous layer was acidified with acetic acid (pH 5–6) and left overnight. The white precipitate obtained was collected by filtration. It was dissolved in boiling water (700 mL). Norite (neutral) was then added and filtered. The filtrate on standing gave 7.6 g of white crystals. The mother liquors on concentration yielded another crop of crystals (1.4 g) for a total yield of **5**, 9.0 g (77%).

Conversion of **8**b *into* **9**

Compound **8***b* was converted by catalytic transfer hydrogenation into **9** by a procedure identical to that described under method A for the conversion of **4** into **5**. Compound **9** was crystallized from ethanol and obtained in an 89% yield.

Condensation of guanine **6**b *with* **1** *to give* **4**

Guanine (3.1 g, 20.5 mmol) and ammonium sulfate (250 mg) were weighed into a 500 mL round-bottom flask. 1,1,1,3,3,3-Hexamethyldisilazane (150 mL) was added and the mixture was heated under reflux with the exclusion of moisture. After 2 days an additional 150 mg of ammonium sulfate was added and reflux was continued. After two days (total 4 days) the solution was clear.

The solvent was removed at reduced pressure and the residue was dissolved in 120 mL of freshly distilled acetonitrile. Tetra-*n*-butylammonium iodide (80 mg) was added, followed by 21.2 mmol of the chloromethylether (compound **1**) in acetonitrile (50 mL). The resulting solution was heated at reflux under nitrogen for 15 h. A thin-layer chromatogram in ethyl acetate – methanol (4:1, v/v) showed a major band at R_f 0.30 and a minor band at the origin. The band at 0.30 contains the desired product and the N-7 isomer in a ratio of 7:3 (nmr determination).

The solvents were removed at reduced pressure and the residue was dissolved in 800 mL of dichloromethane. This solution was washed successively with potassium carbonate solution (10% in water, 200 mL) and water (200 mL). The dichloromethane solution was then evaporated to a small volume. Methanol (50 mL) was added and the solvents were removed at reduced pressure. The residue (10 g) was dissolved in dichloromethane (200 mL) and applied directly to a silica gel column (8.5 × 17 cm, 500 g). Elution was carried out using ethyl acetate – methanol (4:1, v/v). Fractions of 20 mL were collected every 15 min. The desired product was contained in fractions 21 to 130. The material contained in these fractions was washed with hot ethyl acetate and then crystallized from ethanol. The N-7 isomer (**8***b*) selectively crystallizes first, followed by a slower crystallization of **4**. In this way 3.64 g of **4** (41%) was obtained. The yield of the N-7 isomer was 1 g (11%).

Acknowledgement

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