*Carbohydrate Research*, 78 (1980) 285–293


# NUCLEOSIDE SYNTHESIS: A SYSTEMATIC STUDY OF THE INFLUENCE OF THE NATURE AND STEREOCHEMISTRY OF D-ALDOPENTOFURANOSES, AND THE EFFECT OF THE SUBSTITUENT AT C-2, IN THE ACID-CATALYZED FUSION-REACTION WITH INDAZOLE

BERNARD L. KAM*, JEAN-LOUIS BARASCUT, AND JEAN-LOUIS IMBACH**

*Laboratoire de Chimie Bio-Organique, Université des Sciences et Techniques du Languedoc, Place Eugène Bataillon, 34060 Montpellier cédex (France)*

(Received July 12th, 1979; accepted for publication, August 18th, 1979)

## ABSTRACT

A systematic study of the acid-catalyzed fusion-reaction is reported. The influence of the nature and stereochemistry of the D-aldopentofuranose and the effect of the substituent at C-2 have been investigated by using indazole as a model heterocycle. The results obtained show that the nature and stereochemistry of the starting, per-*O*-acetylated D-aldopentofuranose have no significant effect upon the product distribution of the acid-catalyzed fusion-reaction. The use of a sugar lacking a participating group at C-2 showed, however, that the absence of participation increases the ratio of *cis*-1′,2′-nucleosides, and the mechanism involved is discussed. In all cases, the results indicated that the distribution of the products is determined by their relative, thermodynamic stabilities.

## INTRODUCTION

The fusion reaction is one of the most recent methods introduced for the synthesis of nucleosides[1]. This technique for formation of a C–N linkage is simple and rapid, and has been extensively used[2–9]. Because few systematic studies of this reaction have been reported[10,11], there are many discrepancies in the literature concerning the obtaining of different anomers and isomers[2–9]. In order to resolve these problems in the aldopentofuranose series, we have investigated the condensation of indazole (**1**) with per-*O*-acetylated D-aldopentofuranoses.

Previously, we reported the influence of *vacuum*, *catalyst*, *temperature*, and *reaction time* upon the distribution of the products generated by the acid-catalyzed fusion-reaction[11], and showed that it is possible to orient the reaction towards the favored formation of a desired isomer. Furthermore, the results permitted us to

*Present address: Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, U.S.A.

**To whom enquiries should be addressed.

isolate for the first time the D-ribofuranosyl derivatives ($\alpha$-*N*-1 and $\beta$-*N*-2) of the benzazole series[12]. Whereas Hosono *et al.*[6] obtained an anomeric mixture of nucleosides by fusion of 2,6-dichloropurine with tetra-*O*-acetyl-$\alpha$-D-ribofuranose, Tolman *et al.*[9] stated that an aldopentofuranose having *cis*-1,2 stereochemistry failed to react in the fusion reaction, even in the presence of a catalyst, and this has been attributed to the absence of anchimeric participation by the acyl group on O-2 of the sugar, in contradiction of the mechanism proposed by Watanabe *et al.*[13] in which the free electron-pair of the ring-oxygen atom can participate in the reaction. These results are not, however, conclusive, as the aldopentofuranoses used by these authors[6,9] were not anomerically pure. In addition, Iwamura *et al.*[10] found that the stereochemistry and nature of the starting sugar, in the pyranose series, have no effect upon the product distribution in the fusion reaction, but these results cannot be directly extrapolated to the furanose series, because of the marked difference in conformation.

In an attempt to increase the usefulness of the acid-catalyzed fusion as a preparative method, we have re-examined the influence of the stereochemistry and nature of the per-*O*-acetylated D-aldopentofuranoses[14] on condensation with indazole, and have investigated the effect of the substituent at C-2 on reactions by use of a pentofuranose lacking a participating group at C-2.

## RESULTS AND DISCUSSION

We had previously shown that indazole nucleosides are readily identified[8] by $^1$H-n.m.r. spectroscopy and that the yields calculated for the reaction products are consistent with those found after purification by column chromatography[11]. These results have been used in the following studies, and the yields reported have been determined, on the basis of n.m.r.-spectral integration, for the crude mixture of reaction products.

### *A. Effect of the stereochemistry*

In our previous study[11] with tetra-*O*-acetyl-$\beta$-D-ribofuranose (**2$\beta$**) (having *trans*-1,2-stereochemistry), we showed the marked effect of the proportion of catalyst used upon the product distribution of the reaction (see Table I, and Fig. 1). We have therefore investigated the condensation of indazole (**1**) with the *cis*-1,2 anomer (**2$\alpha$**) under analogous conditions (10 min at 160°/20 mm Hg) using an increasing proportion of *p*-toluenesulfonic acid as the catalyst. The results are given in Table II and Fig. 2.

As may be seen, the total yield of the reaction increases with increasing proportion of catalyst, and reaches a maximum at 4% of *p*-toluenesulfonic acid. The 2-*N*-substituted nucleosides (curves E of Figs. 1 and 2) attain their maximum with 2.5% of catalyst and then decrease rapidly, whereas the 1-*N*-substituted isomers (curves B) become preponderant in the presence of more than 3% of *p*-toluenesulfonic acid. In both cases, the *trans*-1′,2′ compounds (curves C and F) are the main products.

These results are consistent with those obtained on condensation of indazole

TABLE I

FUSION OF INDAZOLE (**1**) WITH **2β**

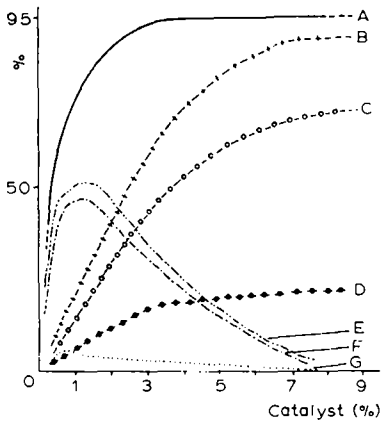
| *Catalyst* (%) | *Total yield* (%) | *Distribution of the products* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Relative to anomers* | | | | *Relative to isomers* | |
| | | α-N-*1* | β-N-*1* | α-N-*2* | β-N-*2* | N-*1* | N-*2* |
| 0.3 | 40 | — | 3 | 5 | 32 | 3 | 37 |
| 0.5 | 52 | 3 | 5 | 5 | 39 | 8 | 44 |
| 0.7 | 61 | 5 | 10 | 5 | 41 | 15 | 46 |
| 1 | 70 | 6 | 14 | 5 | 45 | 20 | 50 |
| 1.5 | 86 | 10 | 23 | 5 | 48 | 33 | 53 |
| 2 | 88 | 12 | 30 | 5 | 41 | 42 | 46 |
| 2.5 | 90 | 14 | 36 | 4 | 36 | 50 | 40 |
| 3.5 | 94 | 17 | 47 | 2 | 28 | 64 | 30 |
| 4 | 95 | 18 | 51 | 2 | 24 | 69 | 26 |
| 5 | 95 | 20 | 58 | — | 17 | 78 | 17 |
| 6 | 95 | 21 | 64 | — | 10 | 85 | 10 |
| 7 | 95 | 22 | 68 | — | 5 | 90 | 5 |
| 8 | 95 | 22 | 70 | — | 3 | 92 | 3 |
| 9 | 95 | 22 | 71 | — | 2 | 93 | 2 |

TABLE II

CONDENSATION OF **1** WITH **2α**

| *Catalyst* (%) | *Total yield* (%) | *Distribution of the products* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Relative to anomers* | | | | *Relative to isomers* | |
| | | α-N-*1* | β-N-*1* | α-N-*2* | β-N-*2* | N-*1* | N-*2* |
| 0.5 | 15 | – | 2 | — | 13 | 2 | 13 |
| 1.2 | 37 | — | 6 | 5 | 26 | 6 | 31 |
| 2 | 62 | 6 | 15 | 6 | 35 | 21 | 41 |
| 2.5 | 76 | 8 | 20 | 8 | 40 | 28 | 48 |
| 3.3 | 88 | 13 | 35 | 6 | 34 | 48 | 40 |
| 3.5 | 92 | 14 | 39 | 6 | 33 | 53 | 39 |
| 5 | 94 | 17 | 56 | 4 | 17 | 73 | 21 |
| 6 | 95 | 17 | 66 | 2 | 10 | 83 | 12 |
| 7 | 95 | 18 | 68 | 2 | 7 | 86 | 9 |

(**1**) with **2β** (see Table I and Fig. 1) and in contradiction to those of Tolman *et al.*[9], according to which **2α** should not react. However, the total yield of nucleosides from **2α** (*cis*-1,2) is highest at a slightly greater proportion of catalyst (4%) than for the *trans*-1,2 anomer (3%). In addition, the maximum yield of 2-*N*-substituted isomers is attained with 2.5% of *p*-toluenesulfonic acid for **2α**, but with 1.4% for **2β**. These results indicate a slight difference in reactivity between the anomers, but also show that, as in the pyranose series[10], the stereochemistry of the starting per-*O*-acetylated



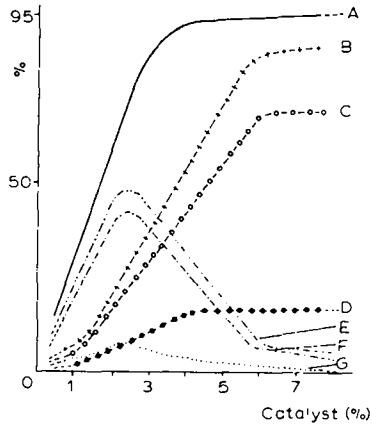




Fig. 1 (left). Fusion of **1** with **2β**. (Product distribution: curve A, total yield of the reaction; B, total *N*-1 isomers; C, β-*N*-1; D, α-*N*-1; E, total *N*-2 isomers; F, β-*N*-2; and G, α-*N*-2.)

Fig. 2 (right). Fusion of **1** with **2α**. (See key to Fig. 1.)

D-aldopentose has no significant effect upon the distribution of the products of the acid-catalyzed fusion-reaction.

In attempting to confirm and generalize these results, we investigated the effect of changing the nature of the aldopentofuranose upon the outcome of this reaction.

*B. Effect of the nature of the sugar*

In order to simplify the n.m.r.-spectral analysis of the mixture of reaction products, we first identified all of the furanosylindazole derivatives* from the tetra-acetates (**7**, **8**, and **9**) of D-xylose, D-lyxose, and D-arabinose, respectively. Then, the reaction was conducted with indazole (**1**) and the tetra-*O*-acetyl-D-pentofuranoses (**2** and **7**–**9**)[14] for 20 min at 155°/20 mm Hg in the presence of 1.4% of *p*-toluenesulfonic acid. The results are presented in Table III, the nucleosides being arranged according to their stereochemistry, namely, *cis*-1′,2′ or *trans*-1′,2′.

In all instances, the total yields of nucleosides from the reaction are comparable. Similarly, for **2**, **7**, and **8**, which were used in pure anomeric form, the yields are consistent within each pair of sugar anomers (see Table III) indicating that the tetra-*O*-acetyl-D-aldopentofuranoses have similar reactivity, and confirming the contention concerning the effect of the stereochemistry. Moreover, the *trans*-1′,2′-nucleosides preponderate in all cases, in agreement with their higher thermodynamic stability[11].

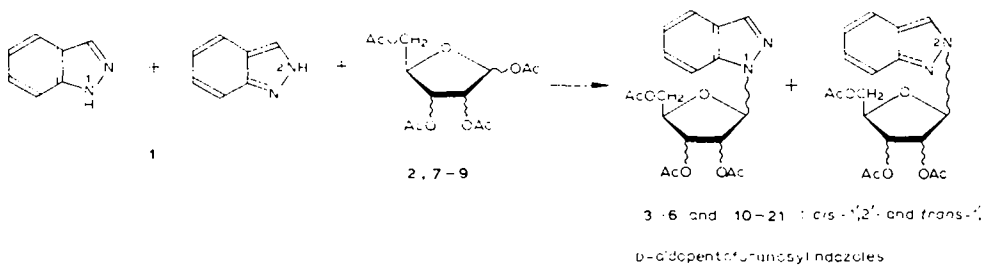
For the mechanism of this reaction, Watanabe *et al.*[13] mentioned possible anomerization of the per-*O*-acetylated sugar during the condensation. To check this possibility, we submitted **2α** and **2β** to the fusion conditions (160°/20 mm Hg). Without any catalyst, no anomerization had occurred up to 30 min. However, in the

*Nucleosides were separated by chromatography on a column of silica gel, and their structures established unambiguously by u.v.- and $^1$H-n.m.r.-spectral analysis.

TABLE III

CONDENSATION OF **1** WITH TETRA-*O*-ACETYL-D-ALDOPENTOFURANOSES

| *Sugar* | *Anomer* | *Total yield* | *Distribution of the products* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Relative to anomers* | | | | *Relative to isomers* | |
| | | | cis-N-*1* | trans-N-*1* | cis-N-*2* | trans-N-*2* | N-*1* | N-*2* |
| **2** | α | 83 | 15 | 30 | 5 | 33 | 45 | 38 |
| | β | 85 | 14 | 33 | 6 | 32 | 47 | 38 |
| **7** | α | 77 | 20 | 25 | 3 | 29 | 45 | 32 |
| | β | 80 | 23 | 30 | 2 | 25 | 53 | 27 |
| **8** | α | 81 | 13 | 40 | 5 | 23 | 53 | 28 |
| | β | 79 | 11 | 38 | 3 | 27 | 49 | 30 |
| **9** | α,β | 78 | 14 | 24 | 5 | 35 | 38 | 40 |




presence of 1.5% of *p*-toluenesulfonic acid, **2α** and **2β** gave an α,β-anomeric mixture, in 2:3 and 2:5 ratio, respectively, in 10 min. Within 30 min, both reached the same anomeric equilibrium mixture (α : β = 1 : 1), indicating similar reactivity of the anomers and suggesting, in agreement with the proposed mechanism[13], that sugars lacking a participating group at C-2 can undergo this reaction. We therefore examined the product distribution from the reaction of such a sugar.

*C. Effect of the substituent at C-2*

1,5-Di-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranose (**22β**) was chosen as a model compound, because it is readily obtained by acetylation of the corresponding diol[16]. In addition, the corresponding indazole nucleosides are well known[8]. The condensation with indazole was conducted during 10 min at 160°/20 mm Hg, with an increasing proportion of *p*-toluenesulfonic acid as the catalyst. The results are given in Table IV and Fig. 3.

As may be seen, in this case, the total yield of nucleosides (curve A, Fig. 3) increases slowly and reaches a maximum at 7% of catalyst. The 2-*N*-substituted isomers (curve E) are maximal with 4% of *p*-toluenesulfonic acid, after which they decrease slowly while the 1-*N*-substituted nucleosides become preponderant (curve B). Stereochemically, low concentrations of catalyst seem to favor the *cis*-1',2'-nucleosides (curves D and G), whereas the *trans*-1',2' anomers become more important with

TABLE IV

CONDENSATION OF **1** WITH **22**/β

| *Catalyst* (%) | *Total yield* (%) | *Distribution of the products* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Relative to anomers* | | | | *Relative to isomers* | |
| | | α-N-*1* | β-N-*1* | α-N-*2* | β-N-*2* | N-*1* | N-*2* |
| 0.3 | 5 | — | — | 3 | 2 | — | 5 |
| 0.7 | 13 | 3 | — | 6 | 4 | 3 | 10 |
| 1.4 | 27 | 3 | 2 | 11 | 11 | 5 | 22 |
| 2.6 | 47 | 8 | 4 | 10 | 25 | 12 | 35 |
| 3.9 | 69 | 16 | 12 | 9 | 32 | 28 | 41 |
| 5 | 86 | 22 | 23 | 8 | 33 | 45 | 41 |
| 6.3 | 94 | 25 | 32 | 7 | 30 | 57 | 37 |
| 7 | 95 | 26 | 33 | 6 | 30 | 59 | 36 |
| 8.7 | 95 | 25 | 37 | 5 | 28 | 62 | 33 |

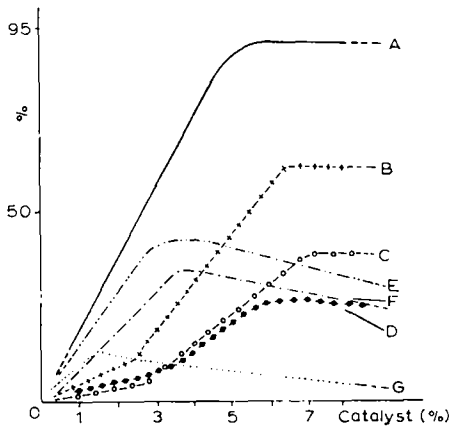



Fig. 3. Condensation of **1** with 1,5-di-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranose (**22**/β). (Key: curve A, total yield of the reaction; B, total *N*-1 isomers; C, β-*N*-1; D, α-*N*-1; E, total *N*-2 isomers; F, β-*N*-2; and G, α-*N*-2.)

larger proportions of catalyst (curves C and F). In addition, compared to the reaction with peracetylated sugar derivatives, there is a general increase in the ratio of *cis*-1′,2′-nucleosides (see Tables I, II, and IV). These results suggest the mechanism presented in Fig. 4. With low proportions of catalyst, *trans*-substitution (SN2 type) might occur. This reaction, similar to those proposed by Hosono *et al.*[6] for fusion without catalyst, will then favor the formation of *cis*-1′,2′-nucleosides, as shown in pathway *A*. With higher proportions of *p*-toluenesulfonic acid, an SN1 type of reaction is more likely to be involved, leading to the formation of an oxonium ion as an intermediate[13]. The allowed attack of the heterocycle from either side of the sugar ring (see pathway *B*) will also favor increased formation of the *cis*-1′,2′-

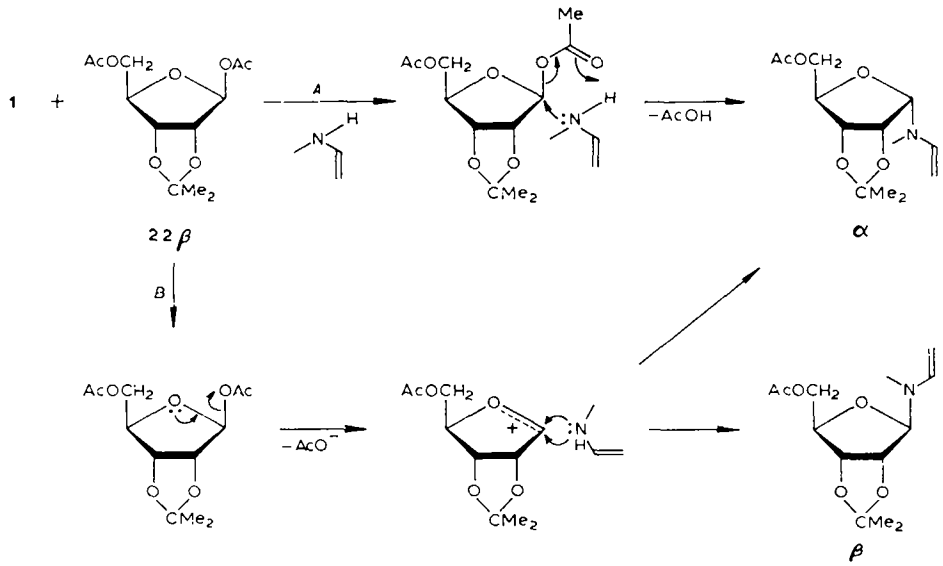


Fig. 4. Proposed mechanism for the reaction of **1** with **22**$\beta$.

nucleosides. However, steric hindrance by the isopropylidene group favors the *trans*-1′,2′ anomers.

The slow increase in the total yield of the reaction suggests that aldopentofuranoses lacking a participating group at C-2 are less reactive than the corresponding 2-acetoxyl derivatives. For both types, the results conclusively demonstrate that the product distribution is determined by their relative thermodynamic stability[11].

## EXPERIMENTAL

*General.* — Merck Kieselgel 60 F 254 fluorescent, aluminum plates were used for t.l.c. Spots were detected with a u.v. lamp, as well as by carbonization after spraying with 10% aqueous sulfuric acid. Merck silica gel 60 (230–400 mesh) was used for column chromatography. $^1$H-N.m.r. spectra were recorded at 60 MHz with a Varian T-60 NMR spectrometer, for solutions in acetonitrile with $Me_4Si$ as the internal, reference standard.

*General conditions for the condensations.* — Equimolar amounts of indazole (**1**) and tetra-*O*-acetyl-D-aldopentofuranose, weighed to $\pm$0.1 mg, were heated in a bath of silicone oil preheated to, and held at, the chosen temperature by a thermostat ($\pm$0.5°). To the molten mixture was then added the desired amount of catalyst, weighed to $\pm$0.01 mg. The percentage of catalyst is expressed in mol, based upon the indazole. The reaction time was measured from the introduction of the catalyst, and the vacuum applied was 20 mm Hg.

*Calculation of the reaction yields.* — N.m.r. spectra were recorded using a 500-Hz sweep-width. The H-3 and anomeric-proton regions were expanded to 250 Hz,

and were integrated. Yields reported are an average of those found by calculation from both regions, and are given with an error within $\pm 2.5\%$.

*Effect of the stereochemistry: condensation of* **1** *with* **2α**. — Compound **1** (0.12 g, 1 mmol) and the stoichiometric amount of **2α** were heated for 10 min at 160° under a pressure of 20 mm Hg, with increasing amounts of *p*-toluenesulfonic acid. The results are given in Table II.

*Effect of the nature of the sugar: condensation of* **1** *with* **2–7**, *and* **9**. — Compound **1** (0.12 g, 1 mmol) and a stoichiometric amount of tetra-*O*-acetyl-D-aldopentofuranose were heated with 1.4% of *p*-toluenesulfonic acid for 20 min at 155° under a pressure of 20 mm Hg. The results are given in Table III.

*Preparation of 1,5-di-*O*-acetyl-2,3-*O*-isopropylidene-*D*-ribofuranose* (**22**). — 2,3-*O*-Isopropylidene-D-ribofuranose[16] (14 mmol) was acetylated with acetic anhydride–pyridine, and the mixture was co-evaporated with 1:4 (v/v) ethanol–chloroform. The residue was purified by chromatography on a column of silica gel eluted with 1:2 (v/v) chloroform–ethyl ether, to give 2.8 g (73%) of **22β** and **22α** (<5%) as oils. Compound **22β** had $[\alpha]_D^{20}$ −69.7° (*c* 1.78, MeOH); $^1$H-n.m.r.: δ 6.22 (s, H-1), 2.10 and 2.07 (2 s, each 3 H, 2 OAc), and 1.43 and 1.32 (2 s, each 3 H, $CMe_2$).

*Anal.* Calc. for $C_{13}H_{18}O_7$: C, 52.55; H, 6.62. Found: C, 52.67; H, 6.48.

Compound **22α**: $^1$H-n.m.r.: δ 5.85 (d, *J* 4.0 Hz, H-1), 2.17 and 2.15 (2 s, each 3 H, 2 OAc), and 1.57, 1.36 (2 s, each 3 H, $CMe_2$).

*Effect of the substituent at C-2: condensation of* **1** *with* **22β**. — Compound **1** (1 mmol) and the stoichiometric amount of **22β** were heated for 10 min at 160° under a pressure of 20 mm Hg, with increasing amounts of *p*-toluenesulfonic acid. The results are given in Table IV.

ACKNOWLEDGMENTS

We are indebted to Drs. Paul Ortiz de Montellano and R. Stuart Tipson for reviewing this manuscript. We also thank the Caisse Nationale de l'Assurance-Maladie for financial support, and the Ministère de l'Education Nationale of the Voltaic Republic for a Fellowship to B.L.K. This work constituted part of a Ph.D. thesis by B.L.K. defended before a jury of the Université des Sciences et Techniques du Languedoc, Montpellier (France), September, 1976.

REFERENCES

1 T. SATO, T. SHIMADATE, AND Y. ISHIDO, *Nippon Kagaku Zasshi*, 81 (1960) 1440–1442.
2 W. PFLEIDERER AND R. K. ROBINS, *Chem. Ber.*, 98 (1965) 1511–1513.
3 K. IMAI, H. NOHARA, AND M. HONJO, *Chem. Pharm. Bull.*, 14 (1966) 1377–1381.
4 J. T. WITKOWSKI AND R. K. ROBINS, *J. Org. Chem.*, 35 (1970) 2635–2641.
5 K. ANTONAKIS AND F. LECLERC, *Bull. Soc. Chim. Fr.*, 6 (1971) 2142–2144.
6 A. HOSONO, K. FUJII, T. TADA, H. TANAKA, Y. OHGO, Y. ISHIDO, AND T. SATO, *Bull. Chem. Soc. Jpn.*, 46 (1973) 2814–2820.
7 J.-L. BARASCUT, C. TAMBY, AND J.-L. IMBACH, *J. Carbohydr. Nucleos. Nucleot.*, 1 (1974) 77–84.
8 B. L. KAM AND J.-L. IMBACH, *J. Carbohydr. Nucleos. Nucleot.*, 1 (1974) 287–289.

9 D. A. BAKER, R. A. HARDER, JR., AND R. L. TOLMAN, *J. Chem. Soc., Chem. Commun.*, (1974) 167–168.
10 H. IWAMURA, M. MIYAKI, AND T. HASHIZUME, *Carbohydr. Res.*, 27 (1973) 149–156.
11 J.-L. BARASCUT, B. L. KAM, AND J.-L. IMBACH, *Bull. Soc. Chim. Fr.*, 11–12 (1976) 1983–1988.
12 J.-L. BARASCUT, B. L. KAM, AND J.-L. IMBACH, *J. Heterocycl. Chem.*, 14 (1977) 1305–1309.
13 K. A. WATANABE, D. H. HOLLENBERG, AND J. J. FOX, *J. Carbohydr. Nucleos. Nucleot.*, 1 (1974) 1–37.
14 B. L. KAM, J.-L. BARASCUT, AND J.-L. IMBACH, *Carbohydr. Res.*, 69 (1979) 135–142.
15 T. NISHIMURA AND B. SHIMUZU, *Chem. Pharm. Bull.*, 13 (1965) 803–810.
16 (a) P. A. LEVENE AND E. T. STILLER, *J. Biol. Chem.*, 102 (1933) 187–201; (b) R. S. KLEIN, H. OHRUI, AND J. J. FOX, *J. Carbohydr. Nucleos. Nucleot.*, 1 (1974) 265–269.