transition state that makes the propane system so sensitive to small perturbations such as isotope and angular momentum effects. Statistical phase space theory can successfully model the reaction cross section, the isotope effect, and the kinetic energy release distribution if C–H rather than C–C bond activation is assumed to be the initial and rate-limiting step for demethanation of propane.[14]

Acknowledgment. The support of the National Science Foundation under Grants CHE88-17201 (M.T.B.), CHE87-11567 (J.L.B.), and CHE87-96289 (P.B.A.) is gratefully acknowledged. J.L.B. also thanks the donors of the Petroleum Research Fund, administered by the American Chemical Society, and J.B.-L. thanks the National Science Foundation for a postdoctoral fellowship. In addition, we gratefully acknowledge helpful discussions with Drs. Martin Jarrold and Marina Rincon regarding the theoretical calculations.

(14) At higher kinetic energies reaction cross-section data suggest that C–C bond insertion processes may become accessible.

n-Pentenyl Glycosides as Efficient Synthons for Promoter-Mediated Assembly of N-α-Linked Glycoproteins[1]

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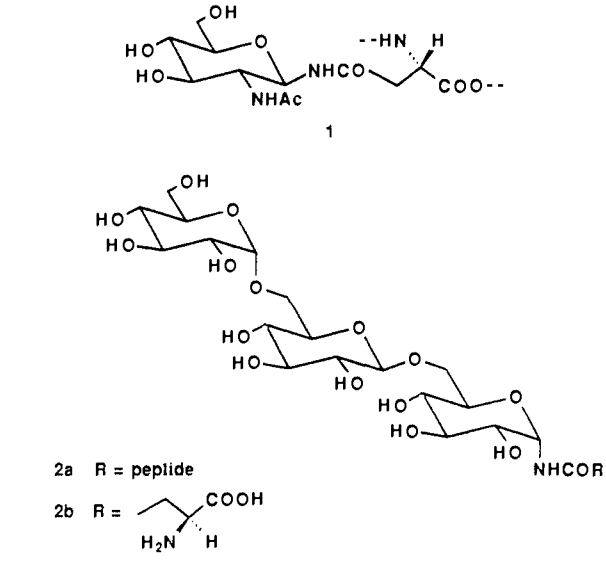
It is now well established that glycoproteins are among some of nature's most widespread bio-regulators, being implicated in a wide variety of vital life processes,[3] and the key roles played by the carbohydrate components have been clearly established.[4] Largely because of modern spectroscopic techniques, daunting problems surrounding elucidation of their structures have been overcome, and their composition, once thought to be hopelessly chaotic, is now known to show certain basic features. Thus, 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine **1** is the common link between the oligosaccharide and polypeptide components,[3a,5] and a variety of synthetic protocols for this N-acyl-β-linkage have been developed.[6,7] The structural elucidation of the nephritogenic glycopeptide **2a**,[8] isolated from rat glomerular basement membrane,[9] was therefore noteworthy in that it contained an α-D-glucopyranose as the glycan "linker" to the amide group of L-asparagine.[10]

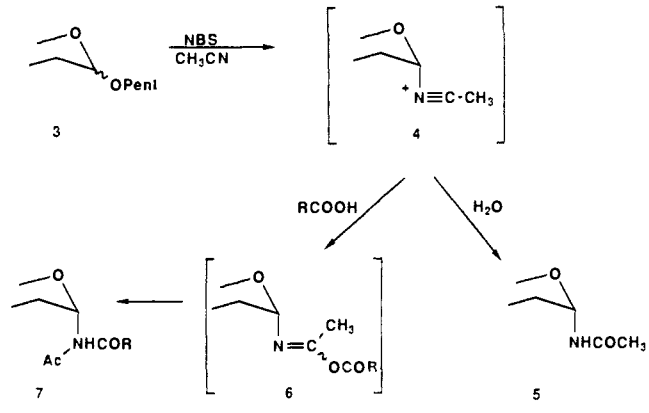
In this communication, we report a synthesis of the crucial segment of **2b**,[11] which demonstrates (a) a novel route to the construction of such α-linked glycopyranosylamides and (b) the use of *N*-iodosuccinimide and trifluoromethanesulfonic acid for reacting "disarmed"[12] *n*-pentenyl glycosyl donors without breaking covalent bonds.[24]

The inspiration for tackling this project came from our recent studies on the oxidative hydrolysis of restrained *n*-pentenyl glycosides in which α-*N*-acetyl glycopyranosyl amines (e.g., **5**) were obtained as major products.[13] Their formation required the

Chart I



Scheme I



(1) This work was supported by grants from the National Science Foundation (CHE 8703916), and Glycomed, Incorporated (Hayward, CA).

(2) (a) A.J.R. is grateful to the Science and Engineering Research Council (UK) for a NATO Postdoctoral Research Fellowship. (b) Present address: IFM/KEMI, University of Linkoping, S-581 83 Linkoping, Sweden.

(3) For reviews see: (a) Montreuil, J. In *Comprehensive Biochemistry*; Neuberger, A., van Deenen, L. L. M., Eds.; Elsevier: Amsterdam, 1982; Vol. 19BII, p 1. (b) Schwarz, R. T.; Datema, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 287. Olden, K.; Bernard, B. A.; Humphries, M. J.; Yeo, T.; Yeo, K.; White, S. L.; Newton, S. A.; Bauer, H. C.; Parent, J. B. *Trends Biochem. Sci. Pers, Ed.* **1985**, *10*, 78. (c) Fukuda, M. *Biochim. Biophys. Acta* **1984**, *780*, 119. (d) Springer, G. F.; Desai, P. R.; Murthy, M. S.; Scanlon, E. F. *ACS Symp. Ser.* **1978**, *80*, 311. (e) Ofek, I.; Beachey, E. H.; Sharon, N. *Trends Biochem. Sci. Pers. Ed.* **1978**, *3*, 159. (f) Feizi, T. *Nature (London)* **1985**, *314*, 53.

(4) (a) Ashwell, G.; Morell, A. G. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1974**, *41*, 99. (b) Olden, K.; Parent, J. B.; White, S. L. *Biochim. Biophys. Acta* **1982**, *650*, 209. (c) von Figura, K.; Hasilik, A. *Trends Biochem. Sci. Pers. Ed.* **1984**, *9*, 29. (d) Race, R. R.; Sanger, R. *Blood Groups in Man*, 6th ed.; Blackwell Scientific: Oxford, 1975.

(5) Johansen, P. G.; Marshall, R. D.; Neuberger, A. *Biochem. J.* **1961**, *78*, 518.

(6) For a recent, up to date review of synthetic methods, see: Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294.

(7) (a) Marks, G. S.; Marshall, R. D.; Neuberger, A. *Biochem. J.* **1963**, *87*, 274. (b) Bolton, C. H.; Jeanloz, R. W. *J. Org. Chem.* **1963**, *28*, 3228. (c) Yamamoto, A.; Miyashita, C.; Tsukamoto, H. *Chem. Pharm. Bull.* **1965**, *13*, 1041. (d) Kunz, H.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 883.

(8) (a) Sawaki, M.; Takeda, T.; Ogiharay, T.; Shibata, S. *Chem. Pharm. Bull.* **1985**, *33*, 5134. (b) Shibata, S.; Nakanishi, H. *Carbohydr. Res.* **1980**, *81*, 345; **1980**, *86*, 316.

(9) (a) Shibata, S.; Miyagawa, Y.; Naruse, T.; Takuma, T. *J. Immunol.* **1969**, *102*, 593. (b) Shibata, S.; Nagasawa, T. *J. Immunol.* **1971**, *106*, 1284.

(10) It is not clear from ref 8a whether L-asparagine or L-glutamine is the glycan–protein linkage. We have decided to adopt the former in developing our methodology given it is the one more usually found in glycopeptides.

(11) For an earlier synthesis of **2b**, see: (a) Ogawa, T.; Nakabayashi, S.; Shibata, S. *Agric. Biol. Chem.* **1983**, *47*, 1213. (b) Ogawa, T.; Nakabayashi, S.; Shibata, S. *Carbohydr. Res.* **1980**, *86*, C7. (c) Takeda, T.; Yoshihiro, S.; Hamada, C.; Fujii, R.; Suzuki, K.; Ogihara, Y.; Shibata, S. *Chem. Pharm. Bull.* **1981**, *29*, 3196.

(12) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583. In response to a comment of a referee, the terms "armed/disarmed" were introduced to diffrentiate the phenomenon from "activated/deactivated" which usually apply to intermediates. The choice was fortuitous since it is now clear that disarmed substrates can be readily activated.

(13) (a) Ratcliffe, A. J.; Mootoo, D. R.; Andrews, C. W.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1989**, *111*, 7661. (b) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans 1* **1989**, 1805.

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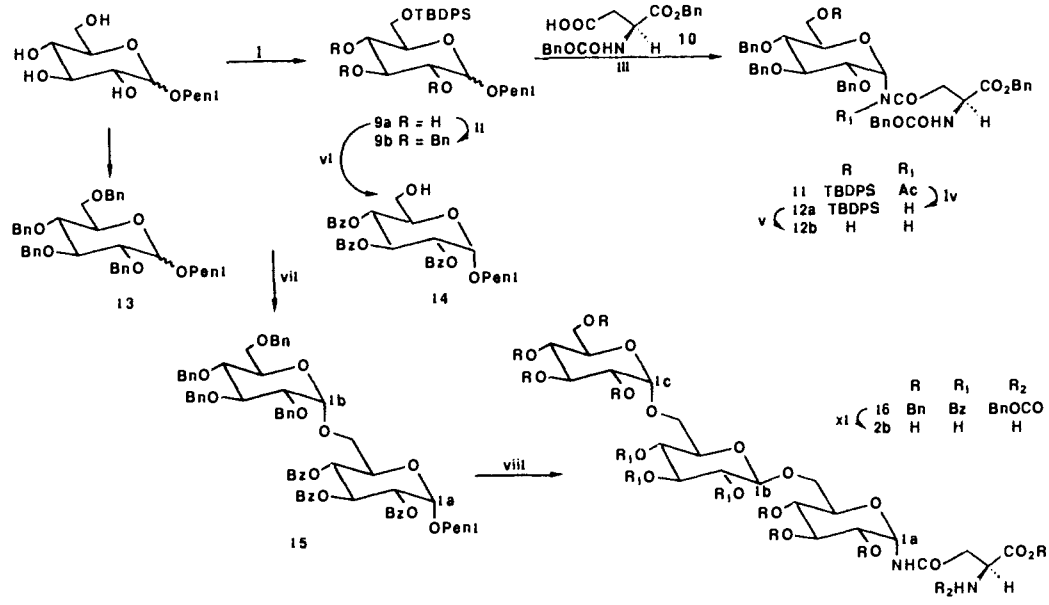
Scheme II



(i) DMAP, Et_3N, CH_2Cl_2, t-$BuPh_2SiCl$, 82%; (ii) DMF, NaH, $N(Bu)_4I$, BnBr, 83%; (iii) **9b**, CH_3CN, NBS, 61%; (iv) DMF, piperidine, 89%; (v) THF, pyridine, HF-pyridine, 95%; (vi) Pyridine, DMAP, BzCl, α+β anomers separated; THF, pyridine, HF-pyridine, 94%; (vii) I(collidine)$_2ClO_4$, CH_2Cl_2. Et_2O, 4Å molecular sieves, 63%; (viii) **12b**, NIS, TfOH, CH_2Cl_2, 4Å molecular sieves, 42%; (ix) NaOH, CH_3OH then 10% Pd/C, EtOH, H_2O, THF, 71%.

occurrence of a Ritter reaction to give the α-acetonitrilium ion **4**, which was trapped by water to give **5**. Therefore, we concluded that, based on the precedents of the laboratories of Sinay[14] and Schmidt,[15] the inclusion of a carboxylic acid in the reaction medium would trap the α-acetonitrilium ion to give the imino anhydride **6**, which would rearrange in situ to the stable α-imide **7**.[16,18]

Accordingly, the differentially protected *n*-pentenyl glycoside **9b** was prepared via standard operations and was dissolved in rigorously dried acetonitrile containing NBS and the known aspartic acid derivative **10**.[19] After 1 h at room temperature, the α-imide **11** was isolated in 61% yield.

We now hoped to effect de-N-acetylation of **11** to obtain **12a**, but contrary to our previous observations with a *N*-acetyl-α-D-glucopyranosylbenzamide analogue,[18] use of sodium methoxide led to complex mixtures. *On the other hand, treatment with piperidine led exclusively to formation of the aspartylamide* ***12a*** *in 89% yield.* Desilylation then gave **12b** with the primary hydroxy group ready for coupling.

The question of the best strategy for assembling the trisaccharide now arose. The tetra-*O*-benzyl and tri-*O*-benzoyl *n*-pentenyl glycosides **13**[25] and **14** were prepared as an armed/disarmed pair. Coupling with iodonium dicollidine perchlorate[20] (IDCP) in ether/dichloromethane (4:1, v/v) proceeded smoothly, in keeping with our previously disclosed procedures,[21] to give the α-linked disaccharide **15** in 63% isolated yield without any evidence of self-coupling of **14**.

Now, in our original protocol for armed/disarmed glycosyl donors, further reaction of **15** would involve replacing the C-2 ester group with an ether.[12] Simple though this strategy is, two discreet chemical operations are required, and these add to the already burdensome logistics of protecting group deployment in oligosaccharide synthesis.

In a striking development, we have found that a disarmed group can be made to react by changing the inorganic source of iodonium ion.[22] Accordingly, the disarmed disaccharide **15** was coupled with **12b** under the agency of iodonium ion, generated in situ from *N*-iodosuccinimide and trifluoromethanesulfonic acid, to give **16**.[23] This promoter therefore greatly simplifies the coupling reaction, which is virtually immediate and allows advantage to be taken of the neighboring group participation of the ester at C-2 of **15** in order to achieve the desired β-linkage.

De-O-benzoylation of **16**, followed by catalytic hydrogenolysis of the crude product, gave the target compound **2b**, exhibiting ^1H and ^{13}C NMR data, as previously reported.[11a]

In conclusion, we have described a short and convergent route to the glycopeptide **2b**, illustrating the use of n-pentenyl glycosides in the stereocontrolled formation of both oligosaccharide and glycopeptide linkages. *The ability to activate a disarmed substrate by simply altering the source of iodonium ion, and without breaking covalent bonds, is considered to be a significant development. Thus, it allows an option to the linkage of monosaccharides to be promoter-specific and complements the substrate-specific strategy of the armed/disarmed protocol.*

Studies to develop this methodology are underway and will be reported in due course.[26]

(14) Pougny, J. R.; Sinay, P. *Tetrahedron Lett.* **1976**, 4073.
(15) Schmidt, R. R.; Michel, J. *J. Carbohydr. Chem.* **1985**, *4*, 141.
(16) Originally, Sinay[14] and Schmidt[15] postulated the intermediacy of β-glucopyranosylacetonitrilium ions, which on trapping with a carboxylic acid led to β-imides. In a reinvestigation of this work[18] we have shown that the acetonitrilium ion adopts an α-configuration[17] with formation of α-imides.
(17) For other carbohydrates α-acetonitriliums see: (a) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244. (b) Pavia, A. A.; Ung-Chhun, N. S.; Durand, J. L. *J. Org. Chem.* **1981**, *46*, 3158.
(18) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans I* **1989**, 147.
(19) Bryant, P. M.; Moore, R. M.; Pimlott, P. J.; Young, G. T. *J. Chem. Soc.* **1959**, 3868.
(20) (a) Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2190. (b) Carlson, H. *Chem. Ber.* **1935**, *68*, 2209. (c) Carlson, H. *Angew. Chem.* **1933**, *46*, 747.
(21) Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. *J. Chem. Soc., Chem. Commun.* **1988**, 823.
(22) We believe that the lower potency of the IDCP is due to the collidine ligands on the iodonium ion. These pyridine-type ligands are necessary to stabilize iodonium perchlorate which is otherwise explosive.[20b,c] NIS/TfOH provides "free" iodonium ion. We have recently found that this can also be achieved with NIS/AgOTf.
(23) The moderate yield of **16** (42%) is undoubtedly a reflection of the known instability of the benzyl carbamate (Cbz) moiety to trifluoromethanesulfonic acid: Yajima, H.; Fujii, N.; Ogawa, H.; Kawatani, H. *J. Chem. Soc., Chem. Commun.* **1974**, 107. The very recent discovery of NIS/AgOTf as an iodonium source[22] might overcome this problem.
(24) Konradsson, P.; Mootoo, D. R.; McDevitt, R. E.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1990**, *270*.
(25) Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1989**, *111*, 540.

Supplementary Material Available: Experimental details for the preparation of compounds **2b**, **9b**, **11**, **12a**, **12b**, **14**, **15**, and **16** (9 pages). Ordering information is given on any current masthead page.

(26) An invention disclosure has been filed to cover the use of *n*-pentenyl glycosides as glycosyl donors.

Facile Aerial Oxidation of the DNA–Base Adduct *N*-(2′-Deoxyguanosin-8-yl)-2-aminofluorene [dG(C8)AF]

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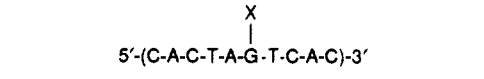
Received October 23, 1989

Recently, in connection with our studies on the synthesis of DNA oligomers containing mutagenic adducts, we were interested in obtaining an oligodeoxynucleotide (**2**) having a deoxyguanosine residue substituted at the C-8 position by an *N*-2-fluorenylamino group.

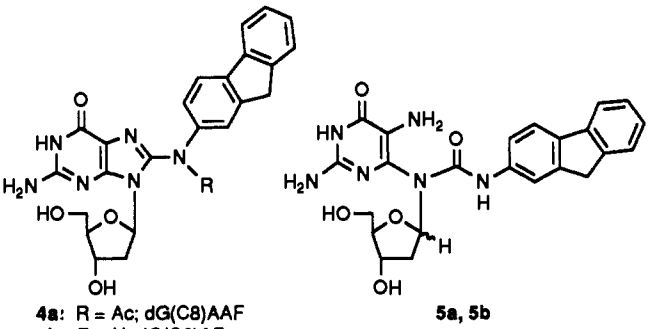


1: X = 8-H
2: X = 8-AF
3: X = 8-AAF

AF = 2-aminofluorene; AAF = *N*-acetyl-2-aminofluorene

Although **3** was easily prepared[1] by the reaction of **1** with *N*-acetoxy-*N*-acetyl-2-aminofluorene, attempts to remove the acetyl group from **3** to obtain **2** using base, invariably led to degraded products. However, the inclusion of a thiol totally prevented[2] the degradation and allowed the isolation of **2** in excellent yield. This result indicated that the degradation is oxidative in nature. Earlier work by Kriek et al.[3,4] had claimed, however, that this degradation of the modified nucleoside *N*-acetyl-*N*-(2′-deoxyguanosin-8-yl)-2-aminofluorene (**4a**) which is present in **3** is solely hydrolytic at alkaline pH. The two products that they isolated were assigned structures **5a** and **5b**, on purely spectroscopic evidence. These conflicting findings led us to reinvestigate this problem both at the level of the oligomers **2** and **3** and at the level of the modified nucleoside **4a**. Although we report studies on **4a** only, in this communication work with both oligomers **2** and **3** has revealed that the corresponding nucleoside residue within the oligomer **3** behaves similarly.[5]

In aqueous solution, in the presence of either a thiol or ascorbic acid or under anaerobic conditions, **4a** is cleanly deacetylated to **4b**, and no degradation could be detected (pH 7–13). This clearly indicates that previously observed transformations are oxidative in nature. Our investigations now show that *the degradative pathway parallels mechanistically the much-studied*[5–8] *oxidation*

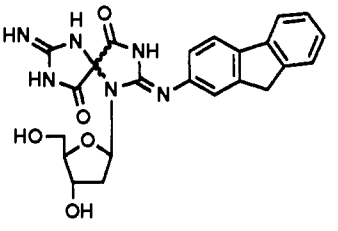


4a: R = Ac; dG(C8)AAF
b: R = H; dG(C8)AF

5a, 5b

of uric acid in alkali. In 0.2 N NaOH, in the presence of air at 75 °C (Kriek and Westra conditions),[4] **4a** rapidly disappears and by HPLC[9] three new compounds arise, which we have designated as ring-opened products (ROP-1, -2, and -3). Under these conditions ROP-3 appears only in the early stages of the reaction as does a fourth peak representing the intermediate deacetylated nucleoside **4b**. Treatment of **4b** under identical conditions also gives rise to the same ring-opened products.

The first products isolated in ~12% yield by HPLC (ROP-1 and ROP-2) occur in a 2:3 ratio[10] and spectroscopically appear to be identical with the two substances **5a** and **5b** first isolated by Kriek and his associates.[4] However, from our own spectroscopic analysis we conclude that most probably these substances are the spirodiastereomers **6a** and **6b**. The ^{1}H NMR and ^{13}C NMR



6a (ROP-1), **6b** (ROP-2)

data[11] unfortunately are not definitive because of the polyaza nature of the substances. Nevertheless the mass spectral results revealed that a good correlation exists between the FAB-MS positive- and negative-ion modes for **6a** and **6b**. Both positive-ion spectra show a peak at *m/z* 463 corresponding to the ion $(M + 1)^+$ whereas the negative-ion spectra show a peak at *m/z* 461 attributable to the ion $(M - 1)^-$. This clearly indicates that the molecular weight of both compounds is 462 daltons (Da), a result that is at variance with the value of 464 found by Kriek and Westra[4] using field-desorption mass spectrometry. The fragmentation patterns in the positive-ion mass spectra are also more easily interpreted in terms of structures **6a** and **6b**. Most significantly, the peak at *m/z* 207 represents the protonated fluorenyl cyanamide (or carbodiimide) ion $FlN{=}C{=}NH_2^+$ rather than the protonated isocyanate ion, $FlN{=}C{=}OH^+$. These new structural assignments make it easy to understand the origin of the diastereoisomeric relationship of **6a** and **6b**, which was assigned originally[4] to (improbable) differences at the anomeric 1′-carbon. It now appears that **6a** and **6b** are (cyclic) reaction path analogues of **7**, a skeletal-rearrangement intermediate postulated to occur along the uric acid–allantoin–uroxanate oxidative pathway.[6] Both

(1) Bases, R.; Mendez, F.; Mendez, L. *Carcinogenesis (London)* **1983**, *4*, 1445–1450.

(2) Stohrer, G.; Osband, J. A.; Alvarado-Urbina, G. *Nucleic Acids Res.* **1983**, *11*, 5093–5102. Stohrer, G.; O'Connor, D. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2325–2329. We found that the quantity of thiol recommended by Stohrer and his associates was insufficient to avoid some oxidation of the aminofluorene adduct. By raising the concentration of mercaptoethanol to 0.25 N, oxidation was completely inhibited at both the monomer and the oligomer levels.

(3) Kriek, E. *Chem.-Biol. Interact.* **1969**, *1*, 3–17. See also: Spodheim-Maurizot, M.; Dreux, M.; Saint-Ruf, G.; Leng, M. *Nucleic Acids Res.* **1979**, *7*, 2347–2356.

(4) Kriek, E.; Westra, J. G. *Carcinogenesis (London)* **1980**, *1*, 459–468.

(5) Brandenberger, H. *Biochim. Biophys. Acta* **1952**, *15*, 108; *Experientia* **1956**, *12*, 208–210; *Helv. Chim. Acta* **1954**, *37*, 641–644.

(6) Brandenberger, H.; Brandenberger, R. H. *Helv. Chim. Acta* **1954**, *37*, 2207–2220.

(7) Poje, M.; Sokolic-Maravic, L. *Tetrahedron* **1986**, *42*, 747–751.

(8) Poje, M.; Sokolic-Maravic, L. *Tetrahedron* **1988**, *44*, 6723–6728.

(9) The degraded monomeric ring-opened products were separated by a reverse-phase column, Bondapak C18 (0.39 × 30 cm, Waters), with a linear gradient of 0.05 M triethylamine acetate, at a flow rate of 1.0 mL/min. Under these conditions the retention times, in minutes, of the relevant compounds, in order of elution, were as follows: dG, 2.7; ROP-2, 11.1; ROP-1, 11.6; ROP-3, 12.6; dG(C8)AAF, 19.9; dG(C8)AF, 23.4.

(10) Neither ROP-1 (**6a**) nor ROP-2 (**6b**) is convertible to ROP-3 on treatment with base, as might be expected, on the basis of their assigned structures.

(11) Sufficient quantities of ROP-3 have not been available for a ^{13}C NMR spectrum determination.

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