Table II. Incorporation of ^{18}O-Labeled Precursors into Lenoremycin[a,b]

[1-^{13}C,1-$^{18}O_2$]acetate[c]				[1-^{13}C,1-$^{18}O_2$]propionate[d]				$^{18}O_2$[e]			
C	^{13}C shift, ppm	$\Delta\delta$, ppm	^{18}O:^{16}O	C	^{13}C shift, ppm	$\Delta\delta$, ppm	^{18}O:^{16}O	C	^{13}C shift, ppm	$\Delta\delta$, ppm	^{18}O:^{16}O
9	68.012	0.024	55:45	1	181.296	0.04	20:80	17	80.939	0.028	20:80
13	108.888	0.032	75:25	5	207.480	0.048	20:80	21	111.091	0.024	15:85
29	98.559	0.024	50:50	11	73.015	0.029	20:80	25	73.113	0.024	20:80
				21	111.073	0.029	15:85	29	98.599	0.032	15:85
								30	64.161	0.024	30:70

[a] $CDCl_3$, 100.63 MHz. [b] Precursors were administered as described in Table I. [c] Sodium [1-^{13}C,1-$^{18}O_2$]acetate, Cambridge Isotope Laboratories, 99 atom % ^{13}C, 95 atom % ^{18}O, diluted to 75% ^{13}C; 143.5 mg (1.75 mmol); 100 mL of culture; 32.9 mg of lenoremycin, av ^{13}C atom % enrichment over natural abundance, 3.4%. [d] Sodium [1-^{13}C,1-$^{18}O_2$]propionate, 54.9% $^{18}O_2{}^{13}$C, 32.17% $^{18}O^{13}$C, 3.6% $^{16}O^{13}$C, diluted to 35% ^{13}C; 100 mg (1.04 mmol); 100 mL culture; 25.4 mg of lenoremycin, av ^{13}C atom % enrichment over natural abundance, 1.9%. [e] Cambridge Isotope Laboratories, 98 atom % $^{18}O_2$; 100 mL of culture; 6.4 mg of lenoremycin, diluted with 1.6 mg of natural abundance lenoremycin.

Having established appropriate conditions for incorporation experiments and identified the basic precursors of lenoremycin, we turned our attention to the origin of the oxygen atoms of the polyether. Thus incorporation of sodium [1-^{13}C,1-$^{18}O_2$]acetate resulted in characteristic ^{18}O-induced isotope shifts[15] of the ^{13}C NMR signals corresponding to C-9, C-13, and C-29 of lenoremycin, indicating that the attached O(4), O(6), and O(10) oxygen atoms are derived from the carboxylate oxygens of acetate. By contrast, no ^{18}O was present at C-17 (Scheme II, Table II). Similarly, incorporation of sodium [1-^{13}C,1-$^{18}O_2$]propionate[16] established that the O(1-2), O(3), O(5), and O(8) oxygen atoms of **2** originate from the carboxylate oxygens of the propionate precursor, based on the observed shifts of C-1, C-5, C-11, and C-21. No shift was observed for C-25. The origin of the remaining oxygen atoms was established by incubating *S. hygroscopicus* in the presence of a 1:4 mixture of $^{18}O_2$ and nitrogen gas.[17] The ^{13}C NMR spectrum of the resulting lenoremycin showed isotopically shifted signals corresponding to C-17 and C-21, C-25 and C-29, and C-30, demonstrating the derivation of O(7), O(9), and O(11) from molecular oxygen.

The above results are completely consistent with the postulated intermediacy of the diepoxy triketone **3**. Thus reductive polyketide chain elongation utilizing the appropriate combination of acetyl CoA (malonyl CoA) and propionyl CoA (methylmalonyl CoA) precursors could give rise to the *all*-(*E*)-triketodiene **5**, in which the individual oxygen atoms, including the C-21 keto oxygen, are all derived from the respective acetate and propionate precursors. Following its release from the polyketide synthetase, the diene **5** is postulated to undergo epoxidation by one or more oxygenases to give the 16*R*,17*R*,24*S*,25*S*-diepoxide **3**. Attack of the C-9 hydroxyl of **3** at the C-13 carbonyl carbon will initiate a cascade of ring closures to generate both spiroketals and the hemiketal ring of lenoremycin. Of particular importance is the derivation of the tetrahydrofuranyl oxygen atom O(8) from propionate, in contrast to the derivation of the analogously located O(8) oxygen atom of monensin from molecular oxygen. Subsequent oxidation at C-30 and glycosylation will complete the biosynthesis of the polyether. Further work on the details of the chain elongation mechanism is in progress.

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(13) Incorporation of [1-^{13}C]acetate led to efficient, indirect enrichment of the majority of the propionate carboxyl-derived carbon atoms of lenoremycin. The sites of labeling were unambiguously assigned by comparison with the results of incorporation of [1-^{13}C]propionate and confirmed by incorporation of [1,2-$^{13}C_2$]acetate. In the latter experiment, the ^{13}C–^{13}C satellites of indirectly enriched propionate-derived carbons, C-1, C-2, and C-3, amounted to ca. 10% of the intensity of the uncoupled but enriched natural abundance peak, in contrast to the ^{13}C–^{13}C satellites corresponding to acetate-derived carbons which constituted ca. 150% of the intensity of the natural abundance peak. The relatively small proportion of intramolecular coupling suggests that the bulk of the acetate-derived propionate units arise via succinyl CoA rather than from rearrangement of endogeneously generated butyrate.[14]

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(16) Synthesized as previously described.[3]

(17) Incubations in the presence of $^{18}O_2$ were carried out by a modification of the previously described apparatus[3] in which the proportion of oxygen to nitrogen was maintained at 1:4 by continual replenishment of oxygen as it was consumed. This technique also allowed the metabolic consumption of oxygen to be monitored continuously.

Anomalous Nitration of Fluoranthene with Nitrogen Dioxide in Carbon Tetrachloride

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In CH_2Cl_2, NO_2/N_2O_4 cleanly nitrates polycyclic aromatic hydrocarbons (PAH's) and is the method of choice for the synthesis of mononitrated derivatives.[1] The mechanism of the reaction of NO_2/N_2O_4 with PAH in aprotic solvents, however, remains controversial. Mechanisms involving free-radical attack,[2] electron-transfer,[3] and electrophilic substitution[4] have been proposed.

In order to obtain additional insights into the mechanism of reaction of NO_2/N_2O_4 with PAH, we have examined the nitration of fluoranthene, **1**, a nonalternant hydrocarbon. Frontier orbital calculations indicate that the positional reactivity in **1** will vary depending upon the nature of the attacking species;[5-7] for example, the order of reactivity for homolytic attack is predicted to be 3 > 1 > 7 > 8 > 2, while the order for electrophilic attack is expected to be 3 > 8 ≅ 7 > 1 > 2. Thus, **1** may provide a probe for distinguishing between radical and electrophilic substitution pathways.

Experimental data for electrophilic substitution[8] (Table I) affords an order of positional selectivity 3 > 8 > 7 > 1 > 2, in agreement with the theoretical prediction. The only previous data for radical substitution on **1** concerns its nitration by N_2O_5.[9] These data were interpreted in terms of the initial σ-complex

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(2) (a) Titov, A. I. *Tetrahedron* **1963**, *19*, 557-580. (b) A radical mechanism is proposed for the reaction of NO_2/N_2O_4 with perylene in aprotic solvents of low polarity by Eberson et al. (Eberson, L.; Radner, F. *Acta Chem. Scand. B* **1985**, *39*, 357-374).

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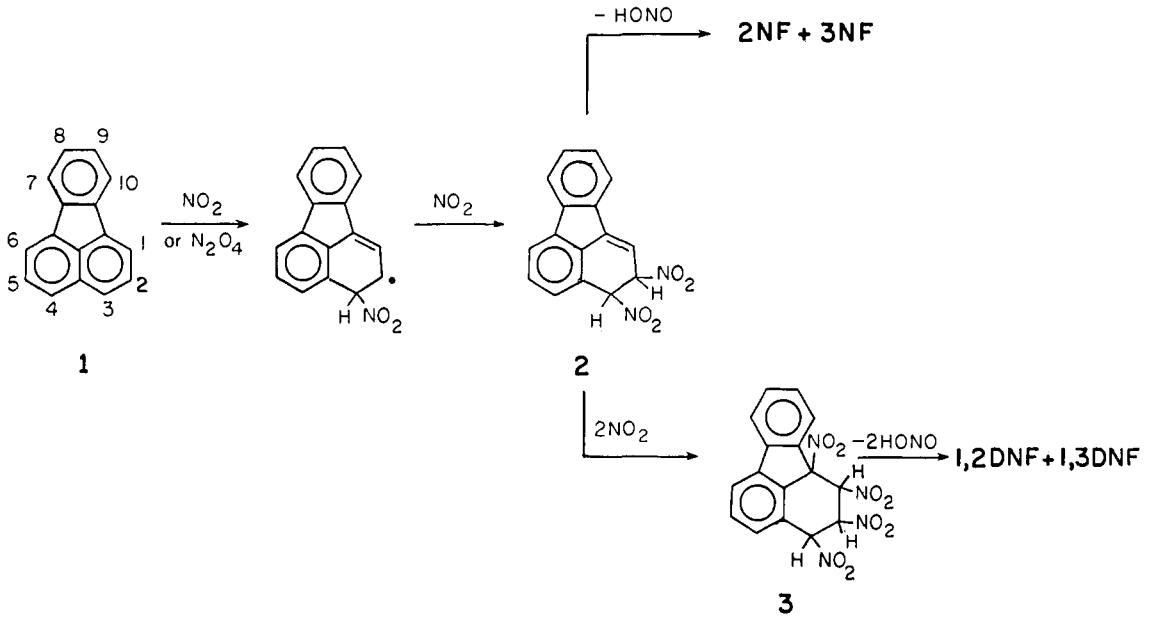
Table I. Nitration of Fluoranthene under Different Conditions

	reagents					
products[a]	NO_2/CCl_4[b]	NO_2/CH_2Cl_2[c]	NO_2/CH_2Cl_2[e]	HNO_3/Ac_2O[g]	HNO_3/Ac_2O[h]	N_2O_5/CCl_4[i]
1NF	1.1 ± 0.3	*d*	2.6 ± 0.3	11.1 ± 3.3	8.6 ± 0.4	
2NF	53.7 ± 2.3		2.3 ± 0.1		*f*	80
3NF	23.7 ± 3.0	63	69.0 ± 1.1	43.5 ± 5.8	49.3 ± 0.1	
7NF	0.7 ± 0.2	*d*	3.2 ± 0.0	18.4 ± 3.9	11.0 ± 0.1	
8NF	1.0 ± 0.1	27	23.0 ± 0.8	27.0 ± 5.8	31.3 ± 0.4	
1,2-DNF	12.3 ± 1.1		*f*		*f*	20
1,3-DNF	7.4 ± 0.9		*f*		*f*	

[a] Percentage distribution, quantified by GC on a 30-m DB-17 capillary column by using flame ionization detector unless specified otherwise. [b] Our work, using conditions specified in ref 10. [c] Reference 1, room temperature. [d] 1NF and 7NF reported together as 10%. [e] Our work, using conditions as in b but with CH_2Cl_2 as solvent. [f] Less than 0.5%. [g] Reference 8, 0 °C; quantified by IR. [h] Our work, conditions as in g but quantification done by GC. [i] Reference 9, 25 °C.

Scheme I



resulting from the attack at the 3-position by the NO_3 radical coupling with NO_2 at the 2-position to give the unstable 3-nitrato-2-nitro-2,3-dihydrofluoranthene which aromatizes by losing a molecule of nitric acid.

We have studied the nitration of **1** under a variety of conditions (Table I). In CH_2Cl_2, NO_2/N_2O_4 displays the same positional order of reactivity as does HNO_3 in Ac_2O, the classical reagent for electrophilic nitration. Thus, NO_2/N_2O_4 appears to react primarily by an electrophilic mechanism in CH_2Cl_2;[1] however, we also have found that a small amount of 2-nitrofluoranthene (2NF) is formed in CH_2Cl_2, which appears to have been previously overlooked.[1]

Since homolytic processes should be facilitated relative to ionic ones as the solvent polarity is decreased, we also studied the nitration of **1** in CCl_4.[10] Both 2NF and 3-nitrofluoranthene (3NF) were isolated as the major products from the reaction mixture and identified by ^1H NMR and GC/MS. Small amounts of 1-nitrofluoranthene (1NF), 7-nitrofluoranthene (7NF), and 8-nitrofluoranthene (8NF) were detected by GC/MS and characterized by comparison of their retention times and fragmentation patterns to those of authentic samples. Traces of a fluoranthene quinone also were detected. Two dinitrofluoranthenes were isolated, and ^1H NMR indicates that disubstitution occurred in one of the rings of the naphthalene-like moiety. One of these disubstituted compounds is 1,2-dinitrofluoranthene (1,2DNF), based on its proton–proton nuclear Overhauser effect (NOE)[9] and confirmed by single-crystal X-ray diffraction analysis.[11] The other dinitro species is 1,3-dinitrofluoranthene (1,3DNF), as indicated by the absence of NOE between the downfield singlet and H-10 with 2NF as a model and by characteristic downfield shifts of the peri and bay protons near the in-plane nitro groups.

Thus, the distinctive features of the nitration in CCl_4 are the formation of much greater yields of 2NF and much lower yields of 8NF than are obtained under electrophilic conditions and the production of modest yields of dinitrofluoranthenes even at low conversions. Furthermore, disubstitution to produce dinitrofluoranthenes always occurs in the same ring, in one case leading to an unexpected 1,2-dinitro species.

Theoretical models involving an intermediate σ-complex[6,7] cannot account for the product distribution unless a second intermediate were formed, as depicted in Scheme I. Attack at the 3-position by NO_2 (or N_2O_4) and subsequent addition of a second NO_2 (perhaps in a cage) leads to the dinitrodihydro intermediate **2**. Intermediate **2** can eliminate a molecule of nitrous acid to form either 2NF or 3NF, determining the mononitrated product specificity. The formation of 2NF may be favored over 3NF due to a small peri interaction between the nitro group and H-4 in the latter case. Alternatively, **2** can react further with 2 more equiv of NO_2 to form the tetranitrotetrahydro intermediate **3**, which can aromatize by losing two molecules of nitrous acid to form 1,2-DNF and 1,3-DNF.[12] This mechanism is similar to

(10) In a typical experiment, 6 mL of a CCl_4 solution of NO_2/N_2O_4 (2.8 mM NO_2 and 45 mM N_2O_4) was added to 2 mL of a 0.15 M solution of fluoranthene under nitrogen at 25 °C. Quantification of products was done by GC by using a flame ionization detector after the evaluation of relative response factors. The product distribution remained constant between 0 and 15% conversion (0–10 h) with a material balance of 90.0 ± 5.3% (triphenylene as internal standard).

(11) Squadrito, G. L.; Fronczek, F. R.; Church, D. F.; Pryor, W. A., to be submitted for publication.

(12) The dinitrofluoranthenes cannot be being formed via heterolytic nitration of the mononitrofluoranthenes, because the latter are expected to be several orders of magnitude less reactive than the parent hydrocarbon; furthermore, a different disubstitution pattern would be expected.

that proposed by Zielinska et al.[9] for the reaction of **1** with N_2O_5; however, in their case the loss of nitric acid is under thermodynamic control so that products with a substituent at the 3-position are not observed.

Thus, **1** allows a distinction between two different mechanisms of reaction of NO_2/N_2O_4. The isomer distribution in CH_2Cl_2 follows the electrophilic substitution pattern predicted from theory, whereas the product distribution in CCl_4 can best be rationalized by a radical mechanism for the nitration.[13] If 2NF is considered as a marker for radical nitration as we suggest, then the homolytic pathway, although not predominant, may be a minor path even in CH_2Cl_2 where some 2NF is formed.

Our results may be of environmental relevance since the uninitiated reaction of NO_2/N_2O_4 with **1** in a solvent of low polarity leads to the formation of nitroaromatics that are potential mutagens.[14] In this regard, 2NF was recently detected among the major nitro-PAH present in ambient air samples,[15-17] and atmospheric radical reactions of **1** initiated by N_2O_5 or hydroxyl radicals have been proposed as possible sources for this pollutant.[15-18]

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(13) One of the referees suggested an alternative mechanism in which a cationic σ-complex is involved; however, we have preliminary data that show that nitrofluoranthene reacts with NO_2/N_2O_4 at a faster rate than does fluoranthene itself, excluding an electrophilic mechanism.

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Asymmetric Alkylation of Chiral β-Lactam Ester Enolates. A New Approach to the Synthesis of α-Alkylated α-Amino Acids

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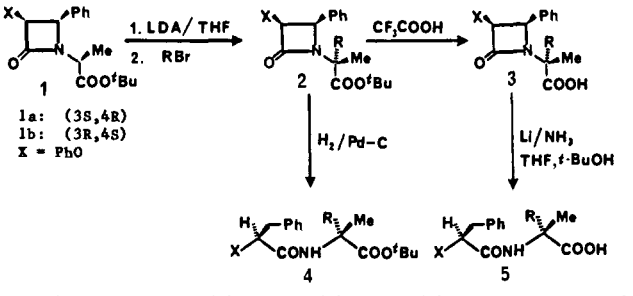
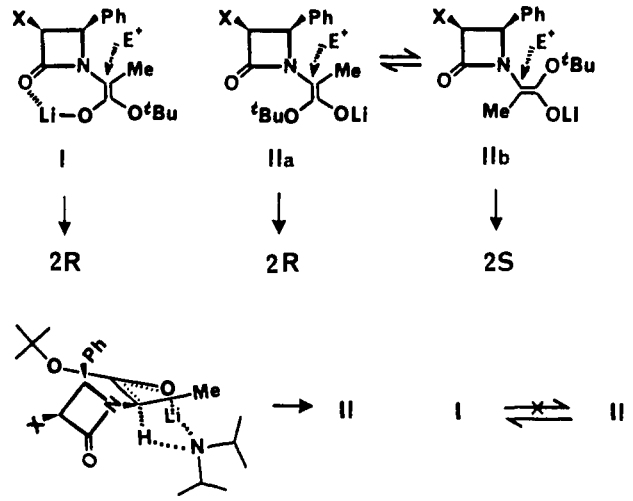
Asymmetric synthesis of nonproteinogenic amino acids with high optical purities has significant value since those amino acids can serve as valuable materials for the study of enzymic reaction mechanisms including enzyme inhibitors. Regarding this approach, Schöllkopf[1] and Seebach[2] reported methods based on bis(lactim) ethers and proline derivatives, respectively. Karady[3] and Williams[4] developed methods based on oxazolidinone and aza-δ-lactone, respectively. We would like to describe here novel approaches to this important synthetic problem through stereoselective alkylations of chiral β-lactam ester enolates followed by the reductive cleavage of the β-lactam rings.[5]

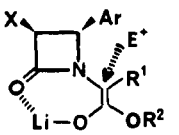
Scheme I



Scheme II



According to our hypothesis, the enolate would form a chelate with the β-lactam oxygen, and then electrophiles should attack from the side opposite to the 4-aryl group.



Thus, β-lactam enolate I was generated by treating β-lactam **1** with LDA (1.0 equiv) in THF at 0-5 °C, and the solution was cooled from -78 to -90 °C. The asymmetric alkylation was carried out by adding an alkyl halide to the enolate I. As shown in Table I, this new asymmetric alkylation proceeded with excellent stereoselectivity.[6] The reductive cleavage of the alkylated β-lactam ester **2** or carboxylic acid **3** thus obtained, through either hydrogenolysis on Pd-C or reduction with dissolving metal ($Li/NH_3/THF/t$-BuOH), gave the corresponding dipeptide derivatives **4** or **5** with high optical purity in excellent yields (Scheme I). The hydrolysis of **4** or **5** with 6 N hydrochloric acid in aqueous

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(6) The absolute configurations of the newly formed chiral quaternary centers were determined based on the chemical correlation and their NMR spectra: for details, see Supplementary Material.

0002-7863/87/1509-6537$01.50/0