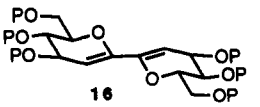
pears that 4 equiv of the arylzinc, with respect to the iodo glucal 5, is the ratio required for an optimum coupling reaction. When 1.3 or 2 equiv of 7e was utilized, there was incomplete consumption of 5 after 24 h. It is also noteworthy that these reactions could be monitored visually since the initially pale yellow solution turns a dark red to black when all of the iodo glucal 5 is consumed.

We were pleased to find that the extension of the reaction to substituted arylboronic acids and arylzinc chlorides was also possible (entries 8–13). Especially interesting, in terms of the potential for preparing sialic acid conjugates, is the facile preparation of the C-furyl glucal 11 (entry 10).[14] Furthermore, the reaction is not limited to the coupling of metalated aromatics since the coupling of 5 and tetravinyltin provided the C-vinyl glucal 15 (entry 14).[15a] The isolated yields of the C-aryl glucals obtained under these mild reaction conditions were also superior to those that we had observed for every analogous example in our earlier work.[15b] Previously, the poorest substrates in the coupling reaction represented by 2 to 6 (eq 1) had been electron-rich aromatics.[1] Thus, the improved yield in the coupling of the anisole derivative (entries 8 and 9)[15b] was gratifying since many of the naturally occurring C-aryl glycosides are oxygen-substituted aromatics.[16] In addition, there was no evidence for the production of the glucal dimer 16 that previously had been the major byproduct



16

(up to 15%) in all of our coupling reactions with stannyl glucal 2.[1] Finally, purification of the glucals 9–15 is more easily accomplished than in the original procedure since the presence of this dimer had, in some cases, hampered chromatographic isolation.[17]

As far as we are aware, this is the first example of the use of the enol ethers of acyl halides as the organic halide partner in a Stille-type coupling reaction with organometallics. We are continuing to explore the scope of this method in the synthesis of naturally occurring C-aryl glycosides as well as in the reactions of other non-carbohydrate derived 1-alkoxy-1-iodoalkenes.

Acknowledgment. We would like to thank Dr. Thomas Keller for a generous gift of the boronic acids 7d and 7f and the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for AIDS Research, and the University of Toronto for financial support of this work.

Supplementary Material Available: Experimental procedure for the preparation of 5 and general procedures for the coupling of 5 and arylboronic acids and arylzinc chlorides, spectral data for 5 and 8–15, and ^{1}H NMR spectra of 5 and 8–15 (23 pages). Ordering information is given on any current masthead page.

(14) Danishefsky has demonstrated that the furan moiety of a C1-furyl glycal is a useful synthetic equivalent of a C1-carboxyl group. Danishefsky, S. J.; DeNinno, M. P.; Chen, S. *J. Am. Chem. Soc.* **1988**, *110*, 3929.

(15) (a) The yield of the C-vinyl glucal 15 (67%, entry 14) is contrasted to the yield of 22% observed by Beau[3b] in the coupling of a 1-stannyl glucal and vinyl bromide. (b) Compare, for example, the yields of compounds 9, 10, 13, and 14 (entries 7, 9, 12, and 13, respectively) to the yields of 6 reported previously[1] that were obtained by using the stannylated glucal 2 and aryl bromides in which R = H (70%), R = 4-MeO (30%), Ar = 1-naphthyl (59%), and R = 2-Me (49%).

(16) Hacksell, U.; Daves, G. D., Jr. *Prog. Med. Chem.* **1985**, *22*, 1.

(17) For example, the C-naphthyl glucal produced in the reaction of 2 and 1-bromonaphthalene (eq 1) had previously been obtained in pure form only in small amounts due to this purification problem.[1] This result is in contrast to the reaction shown in entry 12 in which glucal 13 was isolated in 75% yield.

Synthesis of the Monofluoro Ketone Peptide Isostere

Garry S. Garrett, Thomas J. Emge, Susannie C. Lee, Elaine M. Fischer, Karyn Dyehouse, and John M. McIver*

Corporate Research Division, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati, Ohio 45239

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Summary: A synthetic method for the construction of monofluoro ketone peptide isosteres has been realized. The methodology has been employed in a synthesis of the fluoro ketone replacement for the natural substrate for D,D-carboxypeptidase-transpeptidase.

As part of an ongoing program designed to discover enzyme inhibitors that possess therapeutic potential we were interested in the synthesis of fluorinated ketone derivatives of bioactive peptides. The in vitro inhibition of serine proteases[1] by fluoro ketones that bear a structural resemblance to the natural substrates is well-documented. Fluoro ketone isosteres owe their inhibitory capacity to transition-state stabilization principles[2] that suggest that an enzyme binds the transition state much more strongly than the substrate itself. Similar to the hemiacetal formed by aldehyde inhibitors,[3] fluoro ketones are thought to form a stable hemiketal upon reaction with the active-site serine.[4] In theory, any serine protease can be targeted for inhibition by replacing the amide (Figure 1) located at the scissile bond site of the natural substrate with the ketofluoromethyl group [$C(O)CF_3$,[5] $C(O)CF_2C(O)CFH$], while maintaining the appropriate amino acid residues at adja-

(1) (a) Brady, K.; Abeles, R. H. *Biochemistry* **1990**, *29*(33), 7608. (b) Govardhan, C. P.; Abeles, R. H. *Arch. Biochem. Biophys.* **1990**, *280*(1), 137. (c) Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. *J. Med. Chem.* **1990**, *33*(1), 394. (d) Ueda, T.; Kam, C.; Powers, J. C. *Biochem. J.* **1990**, *265*(2), 539. (e) Allen, K. N.; Abeles, R. H. *Biochemistry* **1989**, *28*(21) 8466. (f) Imperiali, B.; Abeles, R. H. *Biochemistry* **1987**, *26*(14), 4474. (g) Stein, R. L.; Strimpler, A. L.; Edwards, P. D.; Lewis, J. J.; Mauger, R. C.; Schwartz, J. A.; Stein, M. M.; Trainor, D. A.; Wildonger, R. A.; Zottola, M. A. *Biochemistry* **1987**, *26*(10), 2682. (h) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*(13), 3760. (i) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry*, **1985**, *24*(8), 813.

(2) (a) Wolfenden, R. *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 271. (b) Pauling, L. *Chem. Eng. News* **1946**, *263*, 294.

(3) (a) Thompson, R. C. *Biochemistry* **1973**, *12*, 47. (b) Westerik, J. O.; Wolfenden, R. *J. Biol. Chem.* **1972**, *247*, 8195. (c) Evidence for a bound hemiacetal has been provided by X-ray crystallography: Delbaere, L. T. J.; Brayer, G. D. *J. Mol. Biol.* **1985**, *183*, 89.

(4) Brady, K.; Anzhi, W.; Ringe, D.; Abeles, R. H. *Biochemistry* **1990**, *29*(33), 7600.

(5) Although not shown in Figure 1 trifluoromethyl ketones have demonstrated significant potential as inhibitors of serine proteases.

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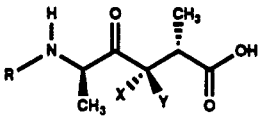
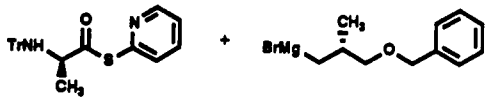
cent sites to ensure adequate primary and secondary binding interactions. Imperiali and Abeles[1h] have demonstrated the inherent variability in binding to chymotrypsin and porcine pancreatic elastase by peptidyl fluoromethyl ketones as a function of the number of fluorine atoms present. Moreover, the type of inhibitory behavior exhibited was also directly related to the degree of fluorination.[6]

Our interest in the antiinfective area led us to investigate the synthesis of fluoro ketone analogues[7] of the peptidyl substrate for D,D-carboxypeptidase-transpeptidase (D,D-CPTPase).[9] In this paper, we discuss the construction of the molecular framework of the monofluoro ketone peptide isostere 1.



1 a - X = F, Y = H
1 b - X = H, Y = F

No general method to synthesize peptidyl monofluorinated ketones with chiral centers intact and the peptide backbone spacing maintained has appeared in the literature.[10] Despite the allure of a 1,4-stereocontrolled approach to the chiral centers embodied in the target isostere **1a,b**, we imagined the combination of two enantiomerically pure pieces might offer the least resistance to the construction of this deceptively simple molecule. An integral part of this plan is the ready access to the reaction partners. The reaction of an *N*-trityl amino acid thiopyridyl ester **2** with an appropriate chiral Grignard reagent **3** seemed to offer the best solution.[11]



2 3

The preparation of bromide **5**[12] from the commercially available (*R*)-(−)-3-hydroxy-2-methylpropionic acid methyl ester (**4**) was straightforward and proceeded as follows (Scheme I). Acid-catalyzed benzylation[13] of **4**, followed by lithium aluminum hydride reduction of the methyl ester and subsequent bromination[14] of the resulting primary alcohol, afforded the chiral bromide **5** in good overall yield. Distillation of the crude product from calcium hydride gave the bromide in suitable form for Grignard formation.[15] With the reaction partners in hand the magnesium-mediated coupling proceeded cleanly to afford the ketone **6** in 78% yield[16] without notable racemization.[17]

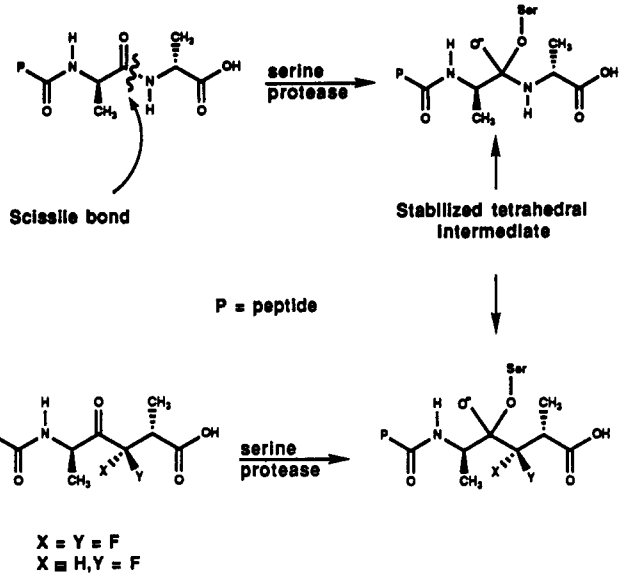
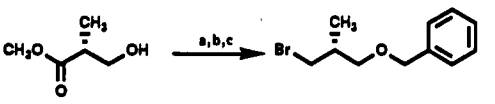


Figure 1. Transition-state stabilization[8] of serine protease.

Scheme I[a]



4 5

[a] Key: (a) $BnOC(NH)CCl_3$, CF_3SO_3H, pentane/CH_2Cl_2, 75%; (b) LAH, Et_2O, 0 °C; (c) Ph_3P, NBS, CH_2Cl_2, 0 °C, combined yield (b + c), 59%.

Critical to the success of our plan was the ability to selectively deprotonate the α' position of **6** to form the enol ether **7** (Scheme II). The enol ether would function as a protecting group for the ketone during several of the successive reaction steps and also serve as a template for an electrophilic fluorination later in the synthesis. At the outset of this work, conditions necessary to selectively deprotonate the α' position were not known.[18] Initial attempts utilizing lithium diisopropylamide (LDA), lithium bis(trimethylsilyl)amide (LBTMSA), and lithium tetramethylpiperidide (LTMP) with a trimethylsilyl chloride (TMS) quench gave varying degrees of both regioisomeric and stereoisomeric products. In contrast, potassium hexamethyldisilazide (KHMDS) afforded a single compound **7a**. To guard against hydrolytic lability we opted

(6) While monofluoro ketones typically exhibit less impressive k_i's than their multiply fluorinated counterparts, they sometimes possess the interesting propensity for exhibiting both competitive, reversible inhibition and irreversible inhibition.

(7) The synthesis of novel difluoro ketone isosteres of the natural substrate for this enzyme recently appeared in a patent application. Schirlin, D.; Jung, M. *European Patent Appl. 88100452.7*, 1987.

(8) Much has often been made of the importance of the tetrahedral geometry in transition-state stabilization. We prefer to draw the tetrahedral intermediate with a full negative charge on oxygen to draw attention to the sometimes overlooked importance of the oxyanion hole in stabilizing the transition state.

(9) This enzyme catalyzes the final step in the formation of the peptidoglycan component of the bacterial cell wall. The peptidoglycan is the major constituent of the cell wall in gram-positive bacteria and constitutes a major portion of the cell wall in most gram-negative organisms.

(10) The stereospecific synthesis of the difluoro ketone peptide isostere is reported: Damon, D. B.; Hoover, D. J. *J. Am. Chem. Soc.* **1990**, *112*(17), 6439.

(11) Johnson, R. L.; Miller, R. B. *Int. J. Peptide Protein Res.* **1983**, *23*, 581.

(12) The enantiomer of this reagent has previously been prepared via asymmetric synthesis. Holladay, M. W.; Salituro, F. G.; Rich, D. H. *J. Med. Chem.* **1987**, *30*(2), 374.

(13) Iversen, T.; Bundle, D. R. *J. Chem. Soc., Chem. Commun.* **1981**, 1240.

(14) Branca, Q.; Fischli, A. *Helv. Chim. Acta* **1977**, *60*, 925.

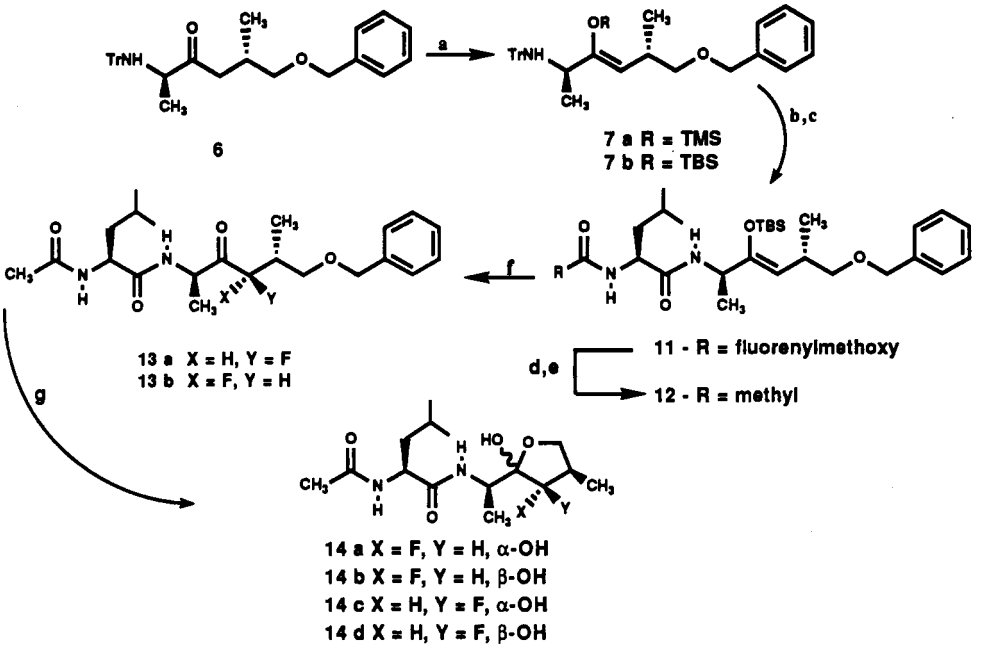
(15) Grignard reaction with **3** has previously been performed on an amino acid aldehyde. See ref 12.

(16) Yields refer to compounds purified by chromatography (flash) or distillation unless otherwise noted. Satisfactory analytical data (^1H NMR, ^{13}C NMR, ^{19}F NMR (when applicable), FAB-MS) were obtained for each new compound synthesized.

(17) An examination of the crude 300-MHz ^1H NMR of the reaction between **2** and **3** revealed the resulting product **6** to be >95% diastereomerically pure, thereby providing a clear indication of the considerable enantiomeric purity of the starting bromide **5** and the lack of racemization in the ensuing organometallic reaction.

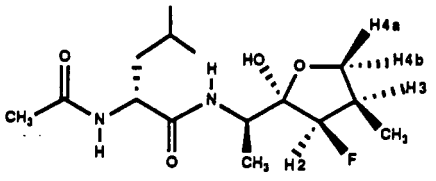
(18) In a related study similar results were obtained. Lubell, W. D.; Rapoport, H. *J. Am. Chem. Soc.* **1988**, *110*(22), 7447.

Scheme II[a]



[a] Key: (a) KHMDS, −78 − 0 °C, then TBSCl, −78 °C (88%); (b) silica gel, $(CO_2H)_2$, CH_2Cl_2, then TEA; (c) Fmoc-L-Leucine, diethyl cyanophosphonate (DEPC), TEA, 66%, steps b + c); (d) piperidine; (e) AcOH, DEPC, TEA, (83% steps d + e); (f) XeF_2, 1,1,2-trichlorotrifluoroethane, (71%); (g) palladium (carbon), ammonium formate, *i*-prOH/AcOH (1:1) (92%).

Table I. Measured Interproton Distances (Å)



14d

	NMR	X-ray
H4a–H4b	1.76	1.76
H2–H3	2.38	2.45
H4b–H3	2.31	2.35
H4a–H3	~3	3.00

for the more stable *tert*-butyldimethylsilyl (TBS) enol ether for the remainder of the synthesis. As expected, reaction of the potassium enolate of **6** with TBSCl also gave a single compound **7b**.[19] Removal of the trityl group from **7b** was accomplished with silica gel and oxalic acid in CH_2Cl_2. For sensitive substrates, this would appear to be the method of choice for removal of tritylamines. Our initial target contained an *N*-acetyl-L-leucine moiety at the amino terminus, and annexation of this portion of the molecule was straightforward and accomplished utilizing standard peptide chemistry. Coupling of the resulting free amine with Fmoc-L-leucine followed by protecting-group removal and acetylation provided the entire carbon framework **12**.[20]

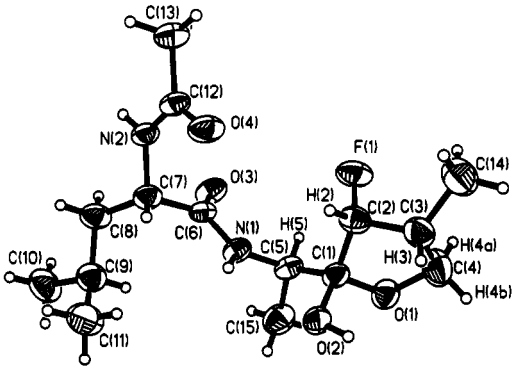
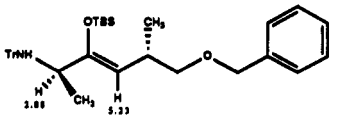


Figure 2. Molecular structure and atomic labeling scheme in the crystal structure of **11d**. Thermal ellipsoids are drawn at the 30% probability level.

We considered several of the existing reagents currently utilized for electrophilic fluorination[21] of enol ethers; however, only XeF_2 offered the safety and ease of handling that we desired. The reaction of XeF_2 with TMS enol ethers and enol acetates has been known to give the α-fluoro ketones for some time but has experienced limited utility.[22] We have discovered that this reaction affords superior yields and cleaner reactions with the TBS enol ether. Reaction of **12** with XeF_2 in a mixture of acetonitrile and 1,1,2-trichlorotrifluoroethane gave the fluoro ketones **13a,b** as a 1:1 mixture of diastereomers in 71% yield.[23]

(19) The stereochemistry of **7** was determined by a difference NOE experiment. NOE's were observed between the vinyl proton at 5.23 ppm and the aromatic protons of the triphenylmethyl group (7.60 ppm) and, more importantly, the methine proton at 2.88 ppm. These data are quite consistent with the isomer shown and argue against the alternative double-bond geometry where the vinyl proton should not be close enough to the proton at 2.88 ppm to observe an NOE. The authors in ref 18 also obtained the *Z* enol ether.



7b

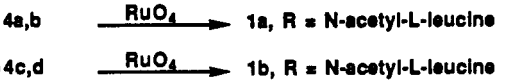
(20) An examination of the crude 300-MHz ^1H NMR indicated the resulting tripeptide analogue was >95% diastereomerically pure.

(21) For a review, see: Rozen, S.; Filler, R. *Tetrahedron* **1985**, *41*(7), 1111.

(22) Tsushima, T.; Kawada, K.; Tsuji, T. *Tetrahedron Lett.* **1982**, *23*(11), 1165.

Hydrogenolysis of the benzyl ether affords four compounds, (14a–d), two sets of which could be separated chromatographically. The ^{13}C, ^{1}H, and ^{19}F NMR of the compounds obtained by chromatography indicated the more polar spot (TLC) contained a 2.5:1 mixture of diastereomers while the more rapid moving spot existed as a 1:1 mixture. Although we were unable to solve the stereochemistry through conventional NMR techniques, we were successful in assigning the relative stereochemistry of a single diastereomer by employing a phase-sensitive 2-D NOESY.[24] This experiment allows for the quantitation of interproton distances by calculating the 3-D volume integrals of the NOESY cross peaks.[25] The calculated interproton distances of the major isomer associated with the more slowly moving pair of diastereomers revealed H_2 is cis to H_3, thus establishing the cis nature of the fluorine/methyl stereochemical relationship. We were able to selectively crystallize a single diastereomer from the 14c/14d mixture and obtain confirmation of our stereochemical assignment. X-ray crystallographic analysis[26] (Figure 2) of this diastereomer (14d) revealed (Table I) a reassuring agreement in the interproton distances.

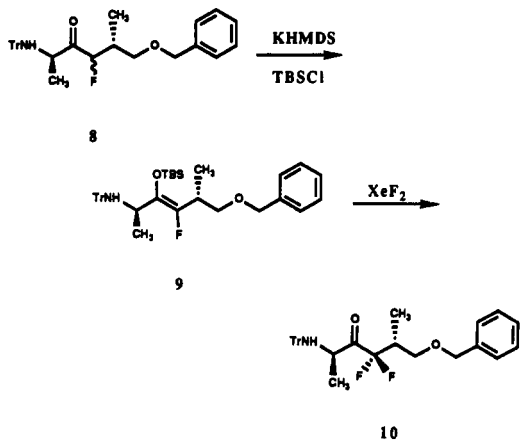
Subsequent confirmation of our successful separation of the fluorine diastereomers was provided by execution of the final step of the synthesis. Oxidation of 14a,b with RuO_4[27] afforded the target isostere 1a (56%) as a single diastereomer. Likewise, oxidation of 14c,d under identical conditions provided the diastereomeric target isostere 1b.



The synthetic blueprint outlined in this report represents the first methodology available to construct monofluoro ketone peptide isosteres with chiral centers intact and the peptide backbone spacing maintained. This method,[28] in addition to novel construction strategies for the synthesis of both difluoro ketone and trifluoromethyl ketone[29] isosteres recently developed in our laboratory, should expedite access to a variety of potent inhibitors and facilitate our understanding of the inhibitory mechanisms[1a,b,f] of this interesting class of molecules.

Acknowledgment. The authors wish to express their appreciation for the contribution of Ms. Ruth Brannon in the preparation of this manuscript. In addition, we also wish to acknowledge the technical assistance of Dr. Charles Eads.

(23) Enol ether 7b can also be fluorinated with this technique to afford the monofluoro ketone 8. However, we were unsure of the compatibility of this functionality with the required subsequent reactions and therefore opted for the reported sequence. Interestingly, we were also able to form the fluoroenol ether 9 from 8 and fluorinate once again to afford the difluoro ketone 10.



(24) We had not actually separated 14c from 14d at this point but the pertinent resonances were resolved in the ^{1}H NMR spectrum.

(25) The phase-sensitive NOESY spectrum data set was recorded into 2048 time-domain data points for 512 t_1 experiments. A mixing time of 250 ms and recycle delay of 6 s were used. A Gaussian multiplication function of 5 Hz was used in the t_1 dimension, while a Gaussian multiplication of 11 Hz and a trapezoidal function of the last 128 points of the free induction decay were applied in the t_2 dimension before Fourier transformation. The final data matrix was 1KX1K.

(26) Single-crystal X-ray diffraction data for 14d, $C_{30}H_{54}F_2N_4O_8$, were collected on a Siemens R3m/E diffractometer with γ = 1.5418 Å (graphite monochromatized), T = 295 K, ω-scan mode (1.0° ranges, 4–30°/min speeds), $3° \leq 2\theta \leq 110°$. Cell data: a = 10.718 (4) Å, b = 17.142 (5) Å, c = 11.220 (2) Å, β = 115.88 (2)°, V = 1854.7 (9) Å^3, D_{calc} = 1.140 g/cm^3; monoclinic, space group $P2_1$, Z = 2; clear, hexagonal rod crystal 0.12 × 0.16 × 0.24 mm. A total of 4909 reflections gave 2399 unique data (R_{int} = 0.02) of which 2033 were observed ($F > 4\sigma$), absorption corrected (μ = 0.709 mm^{-1}, T_{min} = 0.87, T_{max} = 0.92), and used in the structural refinement, yielding R = 0.037 (396 refined parameters), Rw = 0.044 (weights $w = [\sigma^2(F) + 0.0008F^2]^{1/2}$), GOF = 1.15, and difference-map residuals of −0.12 to +0.16 eÅ^{-3}.

(27) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* 1981, *46*(19), 3936.

(28) Given the ready availability of reaction partners similar to 2 and 3 (ref 12), it is not difficult to accept the potential generality of this method for the synthesis of alternative monofluoro ketone isosteric replacements.

(29) New methods for the synthesis of difluoro ketones and trifluoromethyl ketones have been developed in our lab. The synthesis of novel inhibitors of a variety of proteolytic enzymes utilizing this methodology will be reported in due course.

Reaction of Sodium Dithionite Activated Mitomycin C with Guanine at Non-Cross-Linkable Sequences of Oligonucleotides

Brian F. McGuinness,[†] Roselyn Lipman,[‡] Koji Nakanishi,*[,†] and Maria Tomasz*[,‡]

Departments of Chemistry, Columbia University, New York, New York, 10027, and Hunter College, City University of New York, New York, New York 10021

Received April 19, 1991 (Revised Manuscript Received June 13, 1991)

Summary: The reaction of sodium dithionite activated mitomycin C with guanine at non-cross-linkable sequences in oligonucleotides (as well as DNA) yielded a mixed 1″-deoxyguanosine, 10″-sulfonate mitosene derivative, which has implications for published model activation and DNA alkylation studies of this antitumor antibiotic as well as its in vivo mode of action.

The chemotherapeutic agent mitomycin C (MC, 1) must be reduced before it alkylates its putative target, the DNA of a tumor cell.[1] The understanding of the reductive

[†] Columbia University.

[‡] Hunter College, City University of New York.

(1) Szybalski, W.; Iyer, V. N. *Fed. Proc.* 1964, *23*, 946.

0022-3263/91/1956-4826$02.50/0