

# 2'-C-Branched Ribonucleosides. 2. Synthesis of 2'-C- $\beta$ -Trifluoromethyl Pyrimidine Ribonucleosides<sup>1</sup>

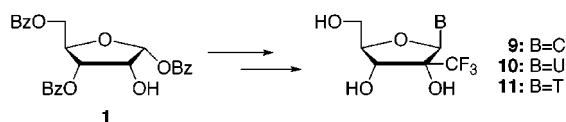
Nan-Sheng Li, Xiao-Qing Tang, and Joseph A. Piccirilli\*

Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biology  
and Department of Chemistry, The University of Chicago, 5841 S. Maryland Avenue,  
MC 1028, Chicago, Illinois 60637

jpicciri@midway.uchicago.edu

Received January 17, 2001

## ABSTRACT



The first synthesis of 2'-C- $\beta$ -trifluoromethyl pyrimidine ribonucleosides is described. 1,2,3,5-Tetra-*O*-benzoyl-2-*C*- $\beta$ -trifluoromethyl- $\alpha$ -D-ribofuranose (3) is prepared from 1,3,5-tri-*O*-benzoyl- $\alpha$ -D-ribofuranose (1) in three steps and converted to 3,5-di-*O*-benzoyl-2-*C*- $\beta$ -trifluoromethyl- $\alpha$ -D-1-ribofuranosyl bromide (5). The 1-bromo derivative (5) is found to be a powerful reaction intermediate for the synthesis of ribonucleosides. The reaction of silylated pyrimidines with (5) in the presence of HgO/HgBr<sub>2</sub> affords exclusively the  $\beta$ -anomers (6–8). Deprotection of (6–8) with ammonia in methanol yields the 2'-*C*- $\beta$ -trifluoromethyl nucleosides (9–11).

2'-*C*-Branched nucleosides exhibit anticancer<sup>2</sup> and antiviral<sup>3</sup> activities, as well as inhibitory activity against several enzymes.<sup>4</sup> For example, 2'-*C*- $\beta$ -methyladenosine resists the action of adenosine deaminase and inhibits the growth of KB cells in culture.<sup>5</sup> 2'-*C*- $\beta$ -Trifluoromethyl nucleosides may be particularly interesting in this regard, as the trifluoromethyl group can enhance the therapeutic properties of bioactive compounds.<sup>6</sup> Additionally, 2'- $\beta$ -trifluoromethyl ribonucleosides may provide important tools for the analysis of RNA structure and function. Specifically, a series of

ribonucleosides analogues in which the 2'- $\beta$ -substituent on the ribose ring is CH<sub>3</sub>, CH<sub>2</sub>F, CHF<sub>2</sub>, or CF<sub>3</sub> might allow a systematic variation of the pK<sub>a</sub> of the 2'-hydroxyl group over a broad range and within a similar structural context. For those enzymes and ribozymes that catalyze RNA strand scission by activating the 2'-hydroxyl group for attack at the adjacent 3'-phosphate diester (such as ribonuclease A or the hammerhead and hairpin ribozymes), this series of nucleosides could reveal a linear free relationship between the catalytic rate and the pK<sub>a</sub> of the nucleophile, thereby providing information about the degree of bond-making between the phosphorus and the nucleophile in the transition state. No existing series of nucleoside analogues possesses these properties. So far, the 2'- $\beta$ -CH<sub>3</sub> derivative is the only member of the series that has been synthesized.<sup>7</sup> The synthesis of 2'-*C*- $\beta$ -trifluoromethyl ribonucleosides is par-

(1) For the initial publication in the series, see: Tang, X.-Q.; Liao, X.-M.; Piccirilli, J. A. *J. Org. Chem.* **1999**, *64*, 747.

(2) (a) Yoshimura, Y.; Satoh, H.; Sakata, S.; Ashida, N.; Miyazaki, S.; Matsuda, A. *Nucleosides Nucleotides* **1995**, *14*, 427 and references therein. (b) Cory, A. H.; Samano, V.; Robins, M. J.; Cory, J. P. *Biochem. Pharmacol.* **1994**, *47*, 365.

(3) Awano, H.; Shuto, S.; Baba, M.; Kira, T.; Shigeta, S.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 367.

(4) (a) Baker, C. H.; Banzon, J.; Bollinger, J. M.; Stubbe, J.; Samano, V.; Robin, M. J.; Lippert, B.; Jarvi, E.; Resvick, R. *J. Med. Chem.* **1991**, *34*, 1879. (b) Ong, S. P.; McFarlan, S. C.; Hogenkamp, H. P. C. *Biochemistry* **1993**, *32*, 11397. (c) van der Donk, W. A.; Yu, G.; Silva, D. J.; Stubbe, J.; McCarthy, J. R.; Jarvi, E. T.; Matthews, D. P.; Resvick, R. J.; Wagner, E. *Biochemistry* **1996**, *35*, 8381.

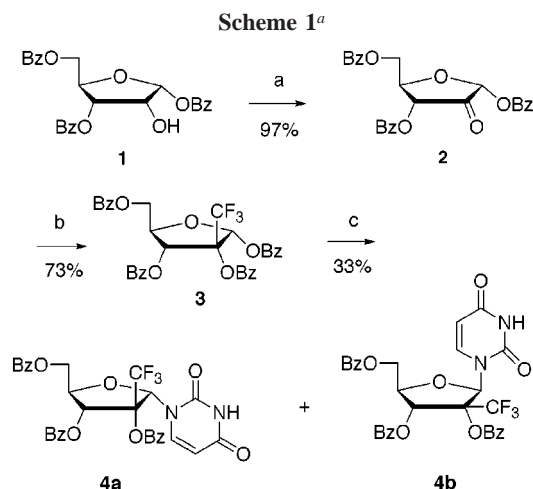
(5) (a) Walton, E.; Jenkins, S. R.; Nutt, R. F.; Zimmerman, M.; Holly, F. W. *J. Am. Chem. Soc.* **1966**, *88*, 4524. (b) Walton, E.; Jenkins, S. R.; Nutt, R. F.; Holly, F. W.; Nemes, M. *J. Med. Chem.* **1969**, *12*, 306.

(6) (a) Welch, J. T. *Selective Fluorination in Organic and Bioorganic Chemistry*; American Chemical Society: Washington, DC, 1991; pp 3–15. (b) Welch, J. T.; Eswarakrishnan, S. *Fluorine in Bioorganic Chemistry*; J. Wiley and Sons: New York, 1991. (c) Hudlicky, M.; Pavlath, A. E. *Chemistry of Organic Fluorine Compounds II*; American Chemical Society: Washington, DC, 1995.

(7) Harry-O'kuru, R. E.; Smith, J. M.; Wolfe, M. S. *J. Org. Chem.* **1997**, *62*, 1754.

ticularly challenging because of the electron-withdrawing and sterically demanding character of the 2'-CF<sub>3</sub> group.<sup>8,9</sup> Here we describe the first synthesis of 2'-C-β-trifluoromethyl ribonucleosides.

Perbenzoylated 2'-C-β-trifluoromethyl-α-D-ribofuranose **3** was synthesized as shown in Scheme 1. Oxidation of 1,3,5-



<sup>a</sup> (a) Dess–Martin periodinane, CH<sub>2</sub>Cl<sub>2</sub>, rt, 24 h; (b) (i) CF<sub>3</sub>SiMe<sub>3</sub>, TBAF(5%), THF, rt, 18 h; (ii) TBAF, rt, 10 min; (iii) BzCl, DMAP, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 5 h; (c) bis(trimethylsilyl)uracil, TMSOTf, CH<sub>3</sub>CN, 120 °C, 3 days, **4a/4b** = 4:96.

tri-*O*-benzoyl-α-D-ribofuranose **1** with Dess–Martin periodinane gave pure 1,3,5-tri-*O*-benzoyl-α-D-2-ketoribofuranose **2** via a modified procedure in 97% yield.<sup>10</sup> Nucleophilic trifluoromethylation of **2** with Ruppert's reagent (CF<sub>3</sub>SiMe<sub>3</sub>) catalyzed by TBAF (5%) in THF<sup>11</sup> followed by desilylation with stoichiometric TBAF and treatment with benzoyl chloride/DMAP/Et<sub>3</sub>N afforded 1,2,3,5-tetra-*O*-benzoyl-2-*C*-β-trifluoromethyl-α-D-ribofuranose **3** in 73% overall yield. <sup>19</sup>F–<sup>1</sup>H NOE experiments indicated that the trifluoromethyl nucleophile added stereoselectively to the β-face of the sugar.<sup>12</sup> When the <sup>19</sup>F nuclei of the trifluoromethyl group were irradiated, we observed strong NOE signals for 1-H (δ 7.42) and 3-H (δ 6.17), suggesting that 1-H, 3-H, and the CF<sub>3</sub> group are on the same side of the ribofuranosyl ring.

(8) It has been reported that the reactions at C-1 of carbohydrates via cationic intermediates are much more difficult to achieve when a trifluoromethyl group is attached to C-2. Logothetis, T. A.; Eilitz, U.; Hiller, W.; Burger, K. *Tetrahedron* **1998**, *54*, 14023 and references therein.

(9) Seebach argued on the basis of van der Waal hemispheres that a trifluoromethyl group is between two and three times larger than a methyl group. See: Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1320. Other assessments suggest that the steric influence of the trifluoromethyl group is closer to that of an isopropyl group or even a *tert*-butyl group. See: (a) Smart, B. E.; Middleton, W. J. *J. Am. Chem. Soc.* **1987**, *109*, 4982. (b) Mosher, H. S.; Stevenot, J. E.; Kimble, D. O. *J. Am. Chem. Soc.* **1956**, *78*, 4374. (c) Ramachandran, P. V.; Teodorovic, A. V.; Brown, H. C. *Tetrahedron* **1993**, *49*, 1725.

(10) Cook, G. P.; Greenberg, M. M. *J. Org. Chem.* **1994**, *59*, 4704.

(11) Prakash, G. K. S.; Krishnamurti, R.; Olah, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 393.

(12) We synthesized 2'-*C*-α-trifluoromethyl-β-D-uridine by the nucleophilic addition of trimethyltrifluoromethylsilane to 3',5'-*O*-(*di-tert*-butylsilyldiyl)-2'-keto-β-D-uridine. Its <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F spectra are different from those of the corresponding 2'-*C*-β-trifluoromethyl-β-D-uridine **10** described in this paper.

The Hilbert–Johnson glycosylation reaction of pyrimidines with peracylated ribose usually proceeds efficiently and stereoselectively at room temperature to yield the β-anomer.<sup>13</sup> However, the glycosylation of bis(trimethylsilyl)uracil with **3** in the presence of either trifluoromethylsilyl trifluoromethanesulfonate or SnCl<sub>4</sub> at room temperature failed to give any 2'-*C*-trifluoromethyluridine. Even after these reactions were refluxed for 1 week in acetonitrile (83 °C), less than 5% of the nucleoside was obtained, though the β-anomer **4b** was formed exclusively. Heating the reaction mixture in a sealed heavy-wall pressure tube at 120 °C for 3 days in the presence of trifluoromethylsilyl trifluoromethanesulfonate improved the yield of nucleoside **4** to 33% and allowed greater recovery of starting material **3** (57%), but both anomers were formed (**4a/4b** = 4:96).<sup>14</sup> At 140 °C for 3 days, the yield of **4** increased to 56% yield, but both the stereoselectivity (**4a/4b** = 14:86) and the recovery of starting material (13%) decreased. At 180 °C, the reaction went to completion more rapidly (<24 h); however, both the yield (21%) and the stereoselectivity decreased significantly (**4a/4b** = 40:60). The stereochemical assignment of **4a** and **4b** as the α and β anomers, respectively, was determined by the <sup>19</sup>F–<sup>1</sup>H NOE experiments. For **4a**, we recorded strong NOE signals for 1'-H (δ 6.87) and 3'-H (δ 6.40) when the <sup>19</sup>F nuclei were irradiated. For **4b**, we observed strong NOE signals for 3'-H (δ 6.23) and 6-H (δ 7.50) upon irradiation of the <sup>19</sup>F nuclei.

The glycosylation reaction with **3** required unusually high temperatures possibly because the CF<sub>3</sub> group hinders the approach of nucleophile<sup>9</sup> and destabilizes the intermediate carbocation at C-1, both by electron withdrawal directly through the σ framework and by weakening the ability of the C-2 benzoyl group to donate electron density to C-1. We explored therefore the possibility of converting the 1-*O*-benzoyl derivative **3** to the more reactive 1-bromo derivative. 2,3,5-Tri-*O*-acyl-D-1-ribofuranosyl halides have been widely investigated as glycosylating agents for the synthesis of ribonucleosides and generally react to give β-anomers exclusively.<sup>15</sup> Giese et al. prepared 2,3,5-tri-*O*-benzoyl-D-1-ribofuranosyl bromide by treating 1,2,3,5-tetra-*O*-benzoyl-α-D-ribofuranose in CH<sub>2</sub>Cl<sub>2</sub> with 33% HBr in acetic acid (0 °C to room temperature).<sup>16</sup> In contrast, **3** was completely unreactive under these conditions, even after the mixture was stirred at room temperature for 9 days. However, heating **3** in a solution of 30% HBr in acetic acid at 80–85 °C for 5 h installed a bromine atom at C-1 but removed the 2-*O*-benzoyl group to give 3,5-di-*O*-benzoyl-2-*C*-β-trifluoro-

(13) (a) Niedballa, U.; Vorbruggen, H. *J. Org. Chem.* **1974**, *39*, 3654, 3664, 3668, 3672. (b) Niedballa, U.; Vorbruggen, H. *J. Org. Chem.* **1976**, *41*, 2084. (c) Vorbruggen, H.; Hofle, G. *Chem. Ber.* **1981**, *114*, 1256.

(14) The anomers were separated with difficulty by silica gel chromatography as described in Supporting Information.

(15) (a) Wittenburg, E. *Chem. Ber.* **1968**, *101*, 1095. (b) Winkley, M. W.; Robins, R. K. *J. Org. Chem.* **1968**, *33*, 2822. (c) Winkley, M. W.; Robins, R. K. *J. Org. Chem.* **1969**, *34*, 431. (d) Currie, B. L.; Robins, R. K.; Robins, M. J. *J. Heterocycl. Chem.* **1970**, *7*, 323. (e) Ozaki, S.; Watanabe, Y.; Hoshiko, T.; Fujisawa, H.; Uemura, A.; Ohrai, K. *Tetrahedron Lett.* **1984**, *25*, 5061. (f) Kissman, H. M.; Pidacks, C.; Baker, B. R. *J. Am. Chem. Soc.* **1955**, *77*, 18. (g) Kessman, H. M.; Child, R. G.; Weiss, M. J. *J. Am. Chem. Soc.* **1957**, *79*, 1185.

(16) Koch, A.; Lamberth, C.; Wetterich, F.; Giese, B. *J. Org. Chem.* **1993**, *58*, 1083.

**Table 1.** Glycosylation of 4-*N*-Benzoyl Cytosine with **5** under Various Conditions

entry	reagents	temp (°C)	time (h)	yield (%) of <b>6</b>
1	(i) <b>5</b> , DMAP, Et <sub>3</sub> N, PhCOCl, CH <sub>2</sub> Cl <sub>2</sub> ; (ii) persilylated base (2.0 equiv), HgO (1.3 equiv), HgBr <sub>2</sub> (0.8 equiv), benzene	80	8	0
2	<b>5</b> , persilylated base (2.7 equiv), HgO (1.3 equiv), HgBr <sub>2</sub> (0.8 equiv), toluene	110	10	48
3	<b>5</b> , persilylated base (1.9 equiv), HgO (1.3 equiv), HgBr <sub>2</sub> (0.8 equiv), benzene	25	62	0
4	<b>5</b> , base (2.0 equiv), HgO (1.3 equiv), HgBr <sub>2</sub> (0.8 equiv), toluene	110	16	0
5	<b>5</b> , persilylated base (2.2 equiv), toluene	110	52	0
6	<b>5</b> , persilylated base (3.0 equiv), HgO (1.5 equiv), benzene	80	14	24
7	<b>5</b> , persilylated base (2.7 equiv), Hg(OAc) <sub>2</sub> (0.9 equiv), toluene	110	10	32
8 <sup>a</sup>	(i) <b>5</b> , HgBr <sub>2</sub> (1.25 equiv), benzene, reflux, 1 h; (ii) persilylated base (2.4 equiv), benzene	80	19	48

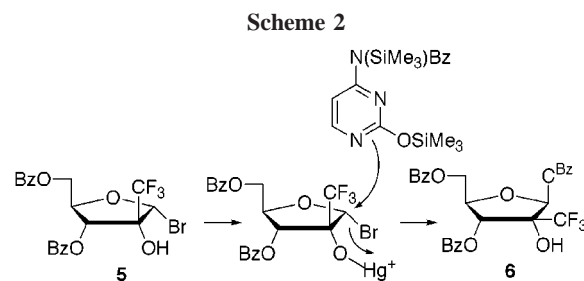
<sup>a</sup> The product contained a 2'-*O*-trimethylsilyl group, which could be removed by TMAF to give **6** in quantitative yield.

methyl- $\alpha$ -D-1-ribofuranosyl bromide **5** in 77% yield. The stereochemistry of **5** was assigned by <sup>19</sup>F-<sup>1</sup>H NOE experiments. We observed strong NOE signals for 1-H ( $\delta$  6.94) and 3-H ( $\delta$  5.68) when the <sup>19</sup>F nuclei were irradiated.

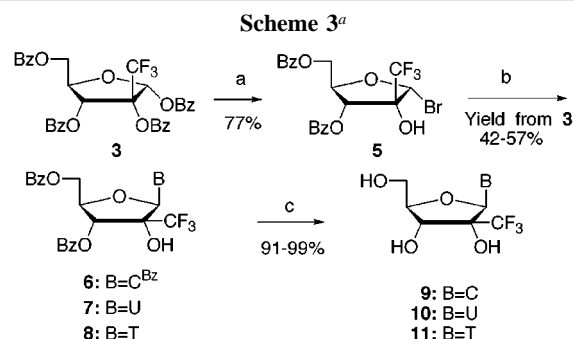
Because the 2-*O*-benzoyl group directs glycosylation reactions, we reintroduced it. Treatment of **5** with benzoyl chloride in the presence of DMAP and triethylamine gave quantitatively 2,3,5-tri-*O*-benzoyl-2-*C*- $\beta$ -trifluoromethyl- $\alpha$ -D-1-ribofuranosyl bromide. Unfortunately, 2,3,5-tri-*O*-benzoyl-2-*C*- $\beta$ -trifluoromethyl- $\alpha$ -D-1-ribofuranosyl bromide was ineffective as a glycosylating agent; reaction in the presence of HgO/HgBr<sub>2</sub><sup>15a</sup> with nucleobases such as persilylated 4-*N*-benzoyl cytosine gave only a trace of product after 8 h at reflux in benzene (Table 1, entry 1). To our surprise, however, the reaction of **5**, which lacks the 2-benzoyl group, with persilylated 4-*N*-benzoyl cytosine gave exclusively the corresponding  $\beta$ -nucleoside derivative, 3',5'-di-*O*-benzoyl-4-*N*-benzoyl-2'-*C*- $\beta$ -trifluoromethyl- $\beta$ -D-cytidine **6** in 48% yield (Table 1, entry 2). Although 3,5-di-*O*-benzoyl-D-1-ribofuranosyl chloride and bromide have been prepared previously,<sup>17</sup> to the best of our knowledge these glycosyl halides have not been used for the synthesis of nucleosides.<sup>18</sup> Consequently, we studied the conversion of **5** to **6** under a variety of reaction conditions (Table 1).

The reaction requires silylation of the nucleobase and the presence of mercury(II), as no reaction occurs with the free nucleobase (Table 1, entry 4) or in the absence of the mercury(II) salt (Table 1, entry 5). Mild heating (refluxing benzene or toluene) also was required (Table 1, entry 3). Interestingly, after 1 h of reflux in benzene in the presence of mercury(II) bromide, **5** remained unchanged in the reaction mixture. However, subsequent addition of the persilylated nucleobase gave in 48% yield the corresponding 2'-*O*-trimethylsilylated product, which can be desilylated by

tetramethylammonium fluoride to give **6** in quantitative yield (Table 1, entry 8). These results suggest that transformation of **5** to **6** may occur via an S<sub>N</sub>2 reaction with the assistance of mercury(II) (Scheme 2).



Direct coupling of crude intermediate **5** (generated in situ from perbenzoylated **3**) with persilylated 4-*N*-benzoylcytosine, uracil, or thymine in the presence of HgO/HgBr<sub>2</sub> gave exclusively the  $\beta$ -nucleoside derivatives **6**, **7**, and **8** in yields of 42%, 47%, and 57%, respectively (Scheme 3). The



<sup>a</sup> 30% HBr in AcOH, 80–85 °C, 5 h; (b) persilylated nucleobases, HgO/HgBr<sub>2</sub>, benzene, or toluene, reflux; (c) NH<sub>3</sub>, MeOH, 4 °C, 2 days.

stereochemistries of **6–8** were confirmed by <sup>19</sup>F-<sup>1</sup>H NOE experiments. For **6**, we observed strong NOE signals for 3'-H

(17) (a) Gorin, P. A. *J. Can. J. Chem.* **1962**, *40*, 276. (b) Ness, R. K.; Fletcher, H. G., Jr. *J. Am. Chem. Soc.* **1956**, *78*, 4710.

(18) A few papers report the reaction of 3,5-di-*O*-acyl-D-1-ribofuranosyl chloride with reactive pyridine derivatives but not with nucleobases. See: (a) Konno, K.; Hayano, K.; Shirahama, H.; Saito, H.; Matsumoto, T. *Tetrahedron* **1982**, *38*, 3281. (b) Freyne, E. J.; Esmans, E. L.; Lepoivre, J. A.; Alderweireldt, F. C. *Carbohydr. Res.* **1980**, *78*, 235. (c) Alderweireldt, F. C.; Kemps, L.; Esmans, E. L.; Dommissie, R. A.; Lepoivre, J. A. *Heterocycles* **1984**, *21*, 795.

( $\delta$  5.85) and 6-H ( $\delta$  8.04) upon irradiation of the  $^{19}\text{F}$  nuclei. For **7** and **8**, we also observed strong NOE signals for 3'-H ( $\delta$  5.96), 6-H ( $\delta$  7.55) of **7** and 3'-H ( $\delta$  5.98) and 6-H ( $\delta$  7.30) of **8** upon irradiation of the  $^{19}\text{F}$  nuclei.

Debenzoylation of **6**, **7**, and **8** with ammonia in methanol at 4 °C for 2 days provided the free 2'-*C*- $\beta$ -trifluoromethyl ribonucleosides **9**, **10**, **11** in yields of 91–99%. CD spectroscopy also confirmed the anomeric configuration of each nucleoside. The CD spectra of **9–11** show a positive cotton effect from 260 to 280 nm as expected for a pyrimidine nucleosides in the  $\beta$ -configuration;<sup>19</sup> the  $\alpha$ -anomers show a negative cotton effect in the same range.

In summary, 2'-*C*- $\beta$ -trifluoromethyl ribonucleosides are challenging compounds to synthesize because of the *cis* relationship between the heterocycle and the large, electron-withdrawing  $\text{CF}_3$  group. By discovering that **5** effectively and stereoselectively glycosylates pyrimidines, we have

---

(19) Miles, D. W.; Inskip, W. H.; Robins, M. J.; Winkley, M. W.; Robins, R. K.; Eyring, H. *J. Am. Chem. Soc.* **1970**, *92*, 3872.

achieved the first synthetic entry into this potentially important class of nucleosides, thereby allowing investigation of their biological and biochemical properties. We plan to convert these trifluoromethyl ribonucleosides to their phosphoramidite derivatives for site-specific incorporation into oligonucleotides via solid-phase synthesis.

**Acknowledgment.** N.S.L is a Research Specialist, X.Q.T. was a Research Associate, and J.A.P. is an Associate Investigator of the Howard Hughes Medical Institute. We thank Dr. A. Bates for help in obtaining NOE spectra and Q. Dai, S. Das, M. Hamm, and J. Schwans for critical comments on the manuscripts.

**Supporting Information Available:** Full experimental and analytical data for compounds **2**, **3**, **4a,b**, and **5–11**;  $^{19}\text{F}$ – $^1\text{H}$  NOE spectra for **3**, **4a,b**, and **5–8**, and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **9–11**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL0155687