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The Shortest Synthetic Route to Puromycin Analogues Using a Modified Robins Approach

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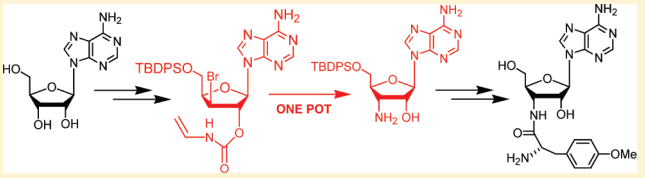
Supporting Information

ABSTRACT: We are reporting on the utility of commercial vinyl isocyanate for a practical synthetic route from adenosine to N^6-bis-demethylpuromycin in seven steps and 65% overall yield. A clean one-pot conversion of 3′-bromo-2′-carbamoyl derivative **8** to 3′-amino-3′-deoxyadenosine derivative **10** is the main feature of this synthetic pathway. This synthesis is the shortest synthetic route toward 3′-(aminoacylamido)deoxyadenosines to date.



The synthesis of puromycin analogues has become a prominent research area ever since the antibiotic puromycin **1** was isolated from a culture broth of *Streptomyces alboniger* by Porter et al.[1] Even though the toxicity associated with puromycin resulted in a very limited use as an antibiotic,[2] puromycin, N^6-bis-demethylpuromycin **2** and other 3′-(L-aminoacylamido)deoxyadenosines (Figure 1) have been extensively used as very useful tools for mechanistic studies on protein biosynthesis[3] because they mimic the nascent peptide accepting 3′-terminus of aminoacyl tRNA[4] and they contain a stable amide bond in the 3′-position. Most of the synthetic routes toward puromycin analogues suffer from numerous steps and low overall yield.[5] In 2001, Samano and Robins reported a synthetic pathway to puromycin by extending the synthetic route to 3′-amino-3′-deoxyadenosine **3**.[6] Even though this method was considered to be the most efficient synthetic route toward puromycin analogues, the very sensitive final debenzylation reaction has been found to give inconsistent results. In 2003 Strazewski and co-workers elaborated on an alternative synthetic pathway to 3′-amino-3′-deoxyadenosine **3** and other analogues of puromycin through a well-known 3′-oxidation/reduction/substitution procedure[7] that helped to overcome the difficulty in the problematic final *N*-debenzylation reaction of the Robins method.[8] However, application of this alternative method involves several disadvantages. Lower initial yield of 2′,5′-bis-*O*-silylated adenosine and the not very convenient separation from its 3′,5′-bis-*O*-silylated regioisomer, the usage of toxic yet irreplaceable chromium(VI) for the oxidation reaction, the limited yield upon scaling up this reaction due to the problematic chromatographic separation from residual chromium salts that may slowly degrade the product, and consequently, the need to proceed with the subsequent reduction reaction within a minimum delay period of time are shortcomings that kept confronting us with the desire to search for alternative routes. We thus turned our interest to the Robins method once again and decided to use commercial vinyl isocyanate, which facilitated the conversion of carbamoyl derivative **8**, to obtain in one pot 3′-amino-3′-deoxyadenosine derivative **10** (Scheme 1).

Adenosine (**4**) was mixed with α-acetoxyisobutyryl bromide[9] in moist acetonitrile to furnish a mixture of *trans*-2′,3′-bromohydrin acetates that were converted into 2′,3′-anhydroadenosine **5** by using high vacuum-dried Dowex 1 × 2 (OH^-) resin in absolute methanol (91% from **4**).[6,10] The primary hydroxyl of **5** was quantitatively protected with a *tert*-butyldiphenylsilyl group to obtain **6**, which underwent, after treatment with synthesized and freshly distilled dimethylboron bromide, clean conversion to 3′-bromo *xylo* isomer **7** in 96% yield.[11]

The replacement of the *N*-benzyl function in the original Robins approach by a more readily cleavable group should be a key factor that could render the Robins strategy the fastest reliable synthetic method toward puromycin analogues. In our initial attempts to replace commercial benzyl isocyanate, *N*-*tert*-butoxycarbonyl isocyanate seemed to be an attractive candidate because the *N*-Boc group could be tracelessly cleaved by a final acid treatment with volatile trifluoroacetic acid in 1,2-dichloroethane (DCE). In our hands, unfortunately, the synthesis of *N*-*tert*-butoxycarbonyl isocyanate was troublesome,[12] its crude purity as determined by IR spectroscopy in DCE (ν NCO st 2236 cm^{-1}; ν CN st 2252 cm^{-1}; ν 2380 cm^{-1}) and the isolated yields varied unreliably. This prompted us to focus on two commercial isocyanates, allyl isocyanate and vinyl isocyanate. Vinyl isocyanate turned out to be the best reagent owing to the ideal combination of its high and chemoselective reactivity on secondary hydroxyl groups and the lability

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of the *N*-vinyl group toward hydrolysis under mildly acidic conditions.

Treatment of **7** with vinyl isocyanate and triethylamine furnished 9-[3-bromo-5-*O*-*tert*-butyldiphenylsilyl-3-deoxy-2-*O*-(*N*-vinylcarbamoyl)-β-D-xylofuranosyl]adenine (**8**) in 96% isolated yield.[6] A one-pot conversion of carbamoyl derivative **8** through **9** to 3′-amino-3′-deoxyadenosine derivative **10** in 93% isolated yield was accomplished by the addition of sodium hydride at −20 °C followed by aqueous-methanolic NaOH and, ultimately, HCl. The cyclized intermediate **9** that forms during the course of this one-pot conversion was isolated and characterized by HRMS (calcd for $C_{29}H_{33}N_6O_4Si$ 557.2333 found 557.2315) and NMR spectroscopy (SI). Thus, the use of vinyl isocyanate in this synthetic pathway resulted in the shortest synthetic pathway for the synthesis of **10** that can be used as a versatile intermediate for the synthesis of different 3′-(aminoacylamido)deoxyadenosines by condensation with different amino acids.

10 was condensed with the oxybenzotriazolyl ester of *N*-Fmoc-*O*-methyl-L-tyrosine, which was formed in situ by the treatment of commercial *N*-Fmoc-*O*-Me-Tyr with HOBt and DIC at 0 °C,[13] to furnish compound **11** in an appreciable 91% yield without protecting the N^6-amidine function. The coupled compound was completely deprotected with ethanolic methyl amine followed in situ by ammonium fluoride in warm methanol, to obtain pure N^6-bis-demethylpuromycin **2** in 92% yield.[10d,14]

In conclusion, a modified Robins approach consisting of seven steps furnished N^6-bis-demethylpuromycin in 65% overall yield. The most notable feature of this approach is the one-pot conversion of carbamoyl derivative **8** to 3′-amino-3′-deoxyadenosine derivative **10**, which makes this synthetic pathway the fastest synthetic route toward puromycin analogues with the highest ever overall yield reported. Furthermore, this synthetic route is well suited for the synthesis of other 3′-(L-aminoacylamido)deoxyadenosines that can be used as the 3′-terminal mimic of different aminoacyl tRNAs.

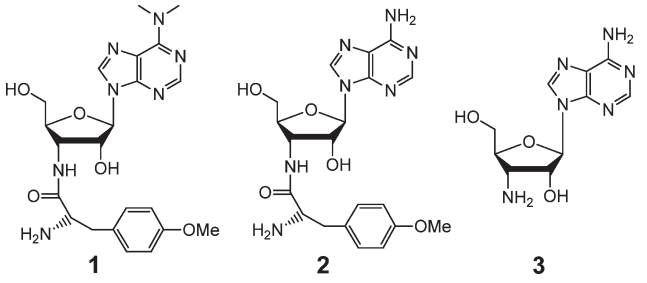


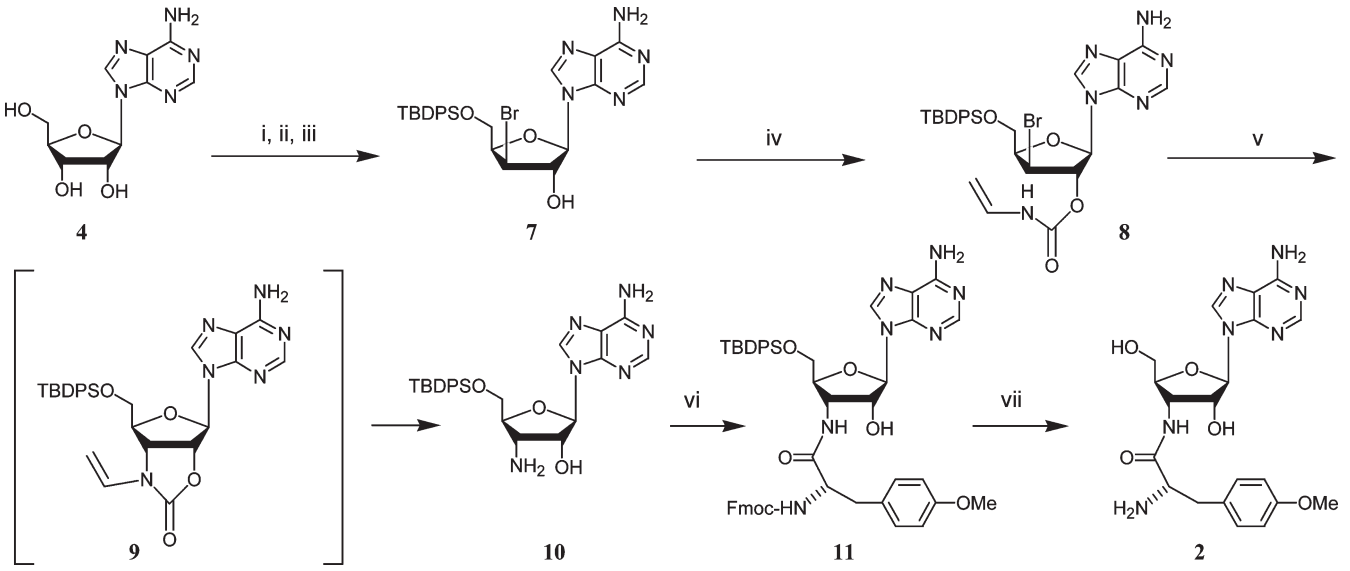
Figure 1. Puromycin (**1**), N^6-bis-demethylpuromycin (**2**), and 3′-amino-3′-deoxyadenosine (**3**).

EXPERIMENTAL SECTION

All nonaqueous reactions were performed in oven-dried glassware under nitrogen. All commercial chemical reagents were used as supplied. The reactions were monitored by thin layer chromatography (TLC), visualized by UV radiation (254 nm) and by spraying with 5% ethanolic H_2SO_4, soaking in ethanolic naphthoresorcinol (2%), and subsequent warming with a heat gun. Column chromatography was performed with flash silica gel (0.04−0.063 mm). High-resolution mass spectrometry (HRMS) was conducted using the electrospray ionization technique (ESI). Compounds were also characterized by ^{1}H NMR and ^{13}C NMR; the assignment of the signals was carried out with the help of DEPT, COSY, and HSQC (see the Supporting Information). Compounds **5**, **6**, and **7** were prepared as described in refs 6 and 10d and characterized by ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, and mass spectrometry.

9-[3-Bromo-5-*O*-*tert*-butyldiphenylsilyl-3-deoxy-2-*O*-(*N*-vinylcarbamoyl)-β-D-xylofuranosyl]adenine (8). Compound **7** (150 mg, 0.26 mmol) was coevaporated with THF (3 × 1 mL) then dissolved in 1:1 anhydrous THF/MeCN (10 mL) under nitrogen. Et_3N (56 μL, 0.40 mmol) and vinyl isocyanate (39 μL, 0.53 mmol) were added to the solution and the mixture was stirred for 48 h at ambient temperature (further reagent was added if necessary

Scheme 1. Synthetic Pathway to N^6-Bis-demethylpuromycin[a]



[a] Reaction conditions: (i) (1) $Me_2C(OAc)COBr$, $MeCN/H_2O$, rt; (2) DOWEX 1 × 2 OH^-, MeOH, rt; (ii) TBDPSCl, pyridine, rt; (iii) Me_2BBr, Et_3N, CH_2Cl_2, −78 °C; (iv) $(CH_2{=}CH)NCO$, Et_3N, THF/MeCN, rt; (v) (1) NaH, THF, −20 °C, (2) NaOH/MeOH/H_2O; rt o/n, (3) aq HCl pH 6 ∼ 6.5, 5min; (vi) Fmoc-Tyr(Me)-OH, HOBt, DIC, 0 °C to rt; (vii) (1) $MeNH_2$/EtOH, rt. (2) NH_4F, MeOH, 55 °C.

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according to TLC). EtOH (500 μL) was added, and stirring was continued for 30 min. Volatiles were evaporated and the residue was chromatographed [SiO_2, CH_2Cl_2/MeOH 100:0 to 93:7 step gradient] to furnish **8** as a white amorphous solid (162 mg, 96%). R_f 0.46 (CH_2Cl_2/MeOH 9:1). HRMS (ESI) calcd for $C_{29}H_{34}BrN_6O_4Si$ 637.1594, found 637.1584.

3′-Amino-5′-*O*-(*tert*-butyldiphenylsilyl)-3′-deoxyadenosine (10). NaH (60% in mineral oil; 9 mg, 0.23 mmol) in a round-bottomed flask was rinsed with hexane (3 × 1 mL), anhydrous THF (3 mL) was added, and the suspension was cooled to −20 °C under N_2. A solution of **8** (122 mg, 0.18 mmol, coevaporated with 3 × 1 mL of THF) in anhydrous THF (2 mL) was added under N_2 to the NaH suspension, and stirring was continued for 12 h at −20 °C. NaOH (228 mg, 5.7 mmol) in MeOH/H_2O 1:1 (4 mL) was added and the reaction mixture was stirred at rt overnight and monitored by TLC. The mixture was acidified to pH 6 ∼ 6.5 with HCl. After 5min, the solution was filtered, the volatiles were evaporated and the residue was chromatographed [SiO_2, CH_2Cl_2/MeOH 100:0 to 92:8 step gradient] to yield **10** (102 mg, 93%) as a white amorphous solid. R_f 0.25 (CH_2Cl_2/MeOH 9:1). HRMS (ESI) calcd for $C_{26}H_{33}N_6O_3Si$ 505.2384, found 505.2372.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-[*N*-(9-fluorenyl)methoxycarbonyl-*O*-methyl-L-tyrosyl]amido-3′-deoxyadenosine (11). *N*-Fmoc-*O*-Me-L-Tyr (108 mg, 0.26 mmol) and HOBt (35 mg, 0.26 mmol) were coevaporated with anhydrous THF (3 × 1 mL) and dissolved in anhydrous THF (2 mL) and then the solution was cooled to 0 °C under N_2 for 10 min. DIC (32 μL, 0.26 mmol) was added and the reaction mixture was stirred at the same temperature for 15 min. This solution was added slowly to a solution of **10** (95 mg, 0.19 mmol) in anhydrous THF (1 mL) and the reaction mixture was stirred for 4 h at rt, taken up in EtOAc (15 mL), and then washed with sat. $NaHCO_3$ solution (15 mL) and H_2O (15 mL). The organic layer was dried with Na_2SO_4 and evaporated and the residue underwent silica gel column chromatography [SiO_2, CH_2Cl_2/MeOH: 100:0 to 92:8 step gradient] to yield **11** (153 mg, 90%) as a white amorphous solid. R_f 0.44 (CH_2Cl_2/ MeOH 9:1). HRMS (ESI) calcd for $C_{51}H_{54}N_7O_7Si$ 904.3854, found 904.3855.

3′-[*O*-Methyl-L-tyrosyl]amido-3′-deoxyadenosine (2). Compound **11** (137 mg, 0.15 mmol) was dissolved in 33% CH_3NH_2/EtOH (12 mL). The reaction mixture was stirred at rt for 1 h. The solution was concentrated under reduced pressure and coevaporated from $CHCl_3$ (2 × 4 mL). The residue was dissolved in MeOH (5 mL) and ammonium fluoride (30 mg, 0.81 mmol) was added to the solution. The reaction mixture was warmed to 50−55 °C, stirred for 4 h, and monitored by TLC. The volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography [SiO_2, EtOAc/MeOH/H_2O 14:1:0.5, 12:1:0.5, 10:1:0.5, 8:1:0.5, 6:1:0.5, 4:1:0.5] to yield after evaporation compound **2** (62 mg, 92%). R_f 0.10 (CH_2Cl_2/MeOH 9:1). HRMS (ESI) calcd for $C_{20}H_{26}N_7O_5$ 444.1996, found 444.1991.

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR signal listings of the compounds and ^1H NMR, ^{13}C NMR, DEPT, COSY, and HSQC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Porter, J. N.; Hewitt, R. I.; Hesseltine, C. W.; Krupka, G.; Lowery, J.; Wallace, W. S.; Bohonos, N.; Williams, J. H. *Antibiot. Chemother.* **1952**, *2*, 409–410.

(2) Kmetec, E.; Tirpack., A. *Biochem. Pharmacol.* **1970**, *19*, 1493–1500.

(3) (a) Ban, N.; Nissen, P.; Hansen, J.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 905–920. (b) Nissen, P.; Hansen, J.; Ban, N.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 920–930. (c) Bayfield, M. A.; Dahlberg, A. E.; Schulmeister, U.; Dorner, S.; Barta, A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10096–10101. (d) Hansen, J.; Schmeing, T. M.; Moore, P. B.; Steitz, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11670–11675. (e) Green, R.; Lorsch, J. R. *Cell* **2002**, *110*, 665–668. (f) Katunin, V.; Muth, G.; Strobel, S. A.; Wintermeyer, W.; Rodnina, M. V. *Mol. Cells* **2002**, *10*, 339–346. (g) Parnell, K. M.; Seila, A. C.; Strobel, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11658–11663. (h) Rodnina, M. V.; Wintermeyer, W. *Curr. Opin. Struct. Biol.* **2003**, *13*, 334–340. (i) Sievers, A.; Beringer, M.; Rodnina, M. V.; Wolfenden, R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7897–7901. (j) Weinger, J. S.; Parnell, K. M.; Dorner, S.; Green, R.; Strobel, S. A. *Nat. Struct. Mol. Biol.* **2004**, *11*, 1101–1106. (k) Youngman, E. M.; Brunelle, J. L.; Kochaniak, A. B.; Green, R. *Cell* **2004**, *117*, 589–599. (l) Schmeing, T. M.; Huang, K. S.; Kitchen, D. E.; Strobel, S. A.; Steitz, T. A. *Mol. Cells* **2005**, *20*, 437–448. (m) Schmeing, T. M.; Huang, K. S.; Strobel, S. A.; Steitz, T. A. *Nature* **2005**, *438*, 520–524. (n) Trobro, S.; Åqvist, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12395–12400. (o) Huang, K. S.; Weinger, J. S.; Butler, E. B.; Strobel, S. A. *J. Am. Chem. Soc.* **2006**, *128*, 3108–3109. (p) Schroeder, G. K.; Wolfenden, R. *Biochemistry* **2007**, *46*, 4037–4044. (q) Wohlgemuth, I.; Brenner, S.; Beringer, M.; Rodnina, M. V. *J. Biol. Chem.* **2008**, *283*, 32229–32235. (r) Koch, M.; Huang, Y.; Sprinzl, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 7242–7245. (s) Lang, K.; Erlacher, M.; Wilson, D. N.; Micura, R.; Polacek, N. *Chem. Biol* **2008**, *15*, 485–492. (t) Kingery, D. A.; Pfund, E.; Voorhees, R. M.; Okuda, K.; Wohlgemuth, I.; Kitchen, D. E.; Rodnina, M. V.; Strobel, S. A. *Chem. Biol.* **2008**, *15*, 493–500. (u) Voorhees, R. M.; Weixlbaumer, A.; Loakes, D.; Kelley, A. C.; Ramakrishnan, V. *Nat. Struct. Mol. Biol.* **2009**, *16*, 528–533.

(4) Yarmolinsky, M. B.; Haba, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1959**, *45*, 1721–1729.

(5) (a) Baker, B. R.; Schaub, R. E.; Joseph, J. P.; Williams, J. H. *J. Am. Chem. Soc.* **1955**, *77*, 12–15. (b) Suami, T.; Tadano, K.; Ayabe, M.; Emori, Y. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 855–861. (c) Okruszek, A.; Verkade, J. G. *J. Med. Chem.* **1979**, *22*, 882–885. (d) Ozols, A. M.; Azhayev, A. V.; Dyatkina, N. B.; Krayevsky, A. A. *Synthesis* **1980**, 557–559. (e) Bouchu, D.; Abou-Assali, M.; Grouiller, A.; Carret, G.; Pacheco, H. *Eur. J. Med. Chem.—Chim. Ther.* **1981**, *16*, 43–47. (f) Lee, H.; Fong, K. L.; Vince, R. *J. Med. Chem.* **1981**, *24*, 304–308. (g) Vince, R.; Daluge, S.; Brownwell, J. *J. Med. Chem.* **1986**, *29*, 2400–2403. (h) McDonald, F. E.; Gleason, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 6648–6659. (i) Botta, O.; Strazewski, P. *Nucleosides Nucleotides* **1999**, *18*, 721–724. (j) Chapuis, H.; Strazewski, P. *Tetrahedron* **2006**, *62*, 12108–12115. (k) Takatsuki, K.; Ohgushi, S.; Kohmoto, S.; Kishikawa, K.; Yamamoto, M. *Nucleosides, Nucleotides Nucleic Acids* **2006**, *25*, 719–734. (l) Charafeddine, A.; Dayoub, W.; Chapuis, H.; Strazewski, P. *Chem.—Eur. J.* **2007**, *13*, 5566–5584. (m) Okuda, K.; Hirota, T.; Kingery, D. A.; Nagasawa, H. *J. Org. Chem.* **2009**, *74*, 2609–2612.

(6) (a) Robins, M. J.; Miles, R. W.; Samano, M. C.; Kaspar, R. L. *J. Org. Chem.* **2001**, *66*, 8204–8210. (b) Samano, M. C.; Robins, M. J. *Tetrahedron Lett.* **1989**, *30*, 2329–2332.

(7) Robins, M. J.; Hawrelak, S. D.; Hernández, A. E.; Wnuk, S. W. *Nucleosides Nucleotides* **1992**, *11*, 821–834.

(8) Nguyen-Trung, N. Q.; Botta, O.; Terenzi, S.; Strazewski, P. *J. Org. Chem.* **2003**, *68*, 2038.

(9) (a) Greenberg, S.; Moffatt, J. G. *J. Am. Chem. Soc.* **1973**, *95*, 4016–4025. (b) Russel, A. F.; Greenberg, S.; Moffat, J. G. *J. Am. Chem. Soc.* **1973**, *95*, 4025–4030.

(10) (a) Robins, M. J.; Hansske, F.; Low, N. H.; Park, J. I. *Tetrahedron Lett.* **1984**, *25*, 367–370. (b) Hansske, F.; Robins, M. J. *Tetrahedron Lett.* **1985**, *26*, 4295–4298. (c) Robins, M. J.; Wilson, J. S.; Madej, D.; Low, N. H.; Hansske, F.; Wnuk, S. F. *J. Org. Chem.* **1995**, *60*, 7902–7908. (d) Michel, B. Y.; Krishnakumar, K. S.; Strazewski, P. *Synlett* **2008**, *16*, 2461–2464.

(11) (a) Guindon, Y.; Yoakim, C.; Morton, H. E. *J. Org. Chem.* **1984**, *49*, 3912–3920. (b) Guindon, Y.; Therien, M.; Girard, Y.; Yoakim, C. *J. Org. Chem.* **1987**, *52*, 1680–1686. (c) Michel, B. Y. *Synlett* **2008**, 2893–2894.

(12) (a) Le Goff, P.; Dewilde, D.; Tsuboniwa, N.; Urano, S. U.S. Patent 5,386,057, January 31 1995. (b) Speziale, A. J.; Smith, L. R. *J. Org. Chem.* **1965**, *30*, 4303–4305. (c) Speziale, A. J.; Smith, L. R. *J. Org. Chem.* **1963**, *28*, 1805–1811. (d) Speziale, A. J.; Smith, L. R. *J. Org. Chem.* **1962**, *27*, 4361–4365.

(13) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.

(14) Krishnakumar, K. S.; Strazewski, P. *Synlett* **2010**, 1055–1059.