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Efficient Synthesis of an Adenosine A2a Agonist: Glycosylation of 2-Haloadenines and an N^2-Alkyl-6-chloroguanine

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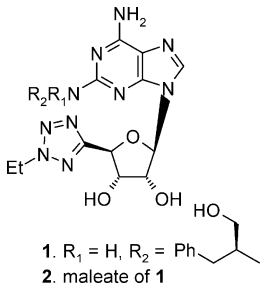
Abstract: A convergent synthesis of adenosine A2a agonist **1** in the form of its maleate salt **2** was achieved. The key step in this approach was the highly selective 9β-glycosylation reaction between 2-haloadenines or an N^2-alkyl-6-chloroguanine and a D-ribose derivative containing a 2-ethyltetrazolyl moiety. Glycosylations of other purine derivatives were also examined, and the methods developed provide efficient access to a variety of adenosine analogues such as 2-alkylaminoadenosines, an attractive class of compounds with antiinflammatory activity.

There is evidence from both in vitro and in vivo studies to indicate that stimulation of adenosine A2a receptors on immune cells produces a series of responses that can be categorized as antiinflammatory.[1,2] It is known that adenosine and some analogues decrease neutrophil adherence to biological substrates and diminish release of tissue-destructive oxidative and nonoxidative products.[2] There has been progressive development of compounds that show increased potency and selectivity as an A2a agonist through addition of 2-alkylamino substituents.[3] Adenosine analogue **1** and its maleate salt **2**, a 2-alkylamino-9-(1-β-ribofuranosyl)adenine containing an ethyltetrazole moiety, are highly selective A2a agonists and have been shown to be efficacious in several animal models of inflammatory disease. Our interest in this class of compounds, and the need for a large quantity of drug substance to support both preclinical and clinical activities, prompted us to search for an efficient synthesis of 9β-2-alkylaminoadenosines such as **1**.



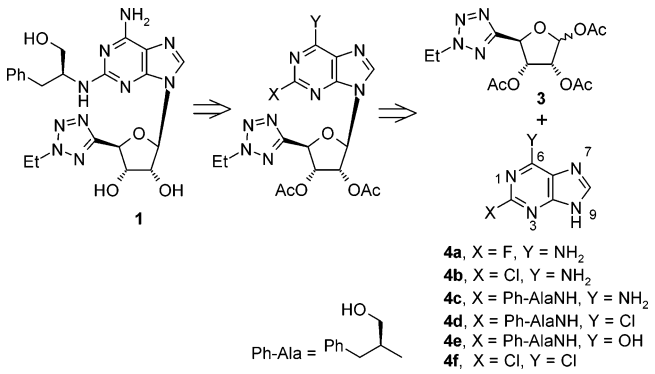


FIGURE 1. Retrosynthetic analysis.

Synthesis of 2-alkylaminoadenosines is generally accomplished through glycosylation of N^2-alkylguanines[4] or 2,6-dihalopurines[5] followed by functionalization of the resultant glycosides. Although there are a number of options for carrying out selective glycosylation of purines, the most attractive with regard to ease of operation and potential for scalability appears to be the conditions developed by Vorbruggen (the silyl Hilbert−Johnson reaction) which employ a silylated nucleoside base and a glycosyl donor.[6] Although often separable by chromatography, significant amounts of undesired N^7 glycosyl and other isomers can be generated from glycosylation of guanine by tetraacetylribose or other furanose derivatives under standard Vorbruggen reaction conditions.[7] In contrast, glycosylation of 2,6-dihalopurines offers increased selectivity of N^9 β-anomer and opportunity to access the desired 2-alkylaminoadenosines.

We now wish to report our studies directed toward the synthesis of 2-alkylaminoadenosine **1** based on glycosylation of several purine derivatives by 5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-2,3,4-triyl triacetate **3** as shown in Figure 1. To our knowledge, there has been little reported on the direct glycosylation of 2-haloadenines **4a** and **4b**,[8,9] 2-alkylaminoadenines such as **4c,** or N^2-alkyl-6-chloroguanines such as **4d** by ribofuranose

(1) For a recent review on medicinal chemistry of adenosine A2a receptor agonists, see: Cristalli, G.; Lambertucci, C.; Taffi, S.; Vittori, S.; Volpini, R. *Curr. Top. Med. Chem.* **2003**, *3*, 387.

(2) For a review on role of A2a adenosine receptors in inflammation, see: Sullivan, G. W.; Linden, J. *Drug. Dev. Res.* **1998**, *45*, 103.

(3) (a) Allen, D. G.; Chan, C.; Cousins, R. P. C.; Cox, B.; Geden; J. V.; Hobbs, H.; Keeling, S. E.; Redgrave, A. J.; Roper, T. D. IV; Xie, S. *PCT Int. Appl.*, WO 9967265, 1999. (b) Cox, B.; Keeling, S. E.; Allen, D. G.; Redgrave, A. J.; Barker, M. D.; Hobbs, H.; Roper, T. D., IV; Geden; J. V. *PCT Int. Appl.*, WO 9828319, 1998. (c) Jarvis, M. F.; Schulz, R.; Hutchison, A. J.; Do, U. H.; Sills, M. A.; Williams, M. *J. Pharmacol. Exp. Ther.* **1989**, 888.

(4) (a) Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* **1984**, *27*, 175. (b) Dudycz, L. W.; Wright, G. E. *Nucleosides Nucleotides* **1984**, *3*, 33. (c) Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2601.

(5) (a) Marchand, A.; Lioux, T.; Mathe, C.; Imbach, J.-L.; Gosselin, G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2249. (b) Martin, P. *Helv. Chim. Acta* **1996**, *79*, 1930.

(6) For reviews, see: (a) Vorbruggen, H. *Acc. Chem. Res.* **1995**, *28*, 509. (b) Vorbruggen, H. *Acta Biochim. Pol.* **1996**, *43*, 25.

(7) (a) Zou, R.; Robins, M. J. *Can. J. Chem.* **1987**, *65*, 1436. (b) Robins, M. J.; Zou, R.; Hansske, F.; Madej, D.; Tyrell, D. L. J. *Nucleosides Nucleotides* **1989**, *8*, 725.

(8) Synthesis of 2-haloadenosines has been reported through microbiological transglycosylation. For 2-fluoroadenine, see: (a) Nakayama, K.; Tanaka, H. Japan Patent 51027755, 1970; *Chem. Abstr.* **1985**, *65*, 2870. For 2-chloroadenine, see: (b) Mikhailopulo, I. A.; Zinchenko, A. I.; Kazimierczuk, Z.; Barai, V. N.; Bokut, S. B.; Kalinichenko, E. N. *Nucleosides Nucleotides* **1993**, *12*, 417. For 2-bromoadenine, see: (c) Huang, M.; Avery, T. L.; Blakley, R. L.; Secrist, J. A., III; Montgomery, J. A. *J. Med. Chem.* **1984**, *27*, 800.

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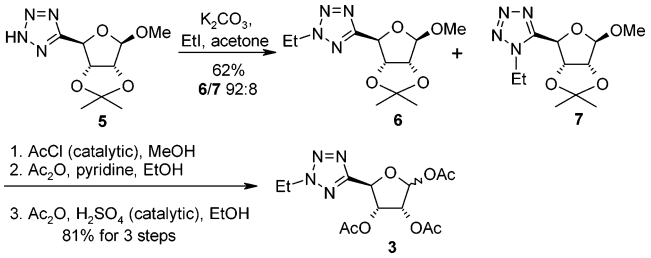
SCHEME 1



derivatives.[10] We were thus interested in investigating the silyl Johnson−Hilbert-type glycosylation of a selection of purines through which a more convergent synthesis of 2-alkylaminoadenosines might be attained. Key considerations for developing a scalable preparation included the N^9/N^7 and β/α selectivity in the glycosylation as well as the downstream efficiency in functionalization of the glycosyl purines.

Triacetate **3** was prepared from tetrazole **5**[11] without the need for chromatography as shown in Scheme 1. Ethylation of tetrazole **5** with EtI and K_2CO_3 resulted in a 2.4:1 ratio of the N^2/N^1 isomers (**6**/**7**).

The undesired N^1 ethyltetrazolyl **7** was largely removed as a solid by fractional crystallization of the crude product from cyclohexane which provided a 92:8 N^2/N^1 mixture of tetrazoles **6** and **7** in 62% yield. The assignment of N^1 ethyl structure **7** was supported by an X-ray crystallographic determination, and through the process of elimination, the N^2 ethyl structure for **6** was assigned. Sequential cleavage of the acetonide, acetylation of the resultant diol, and conversion of the resulting 2,3-di-*O*-acetyl-1-*O*-methyl glycoside to the activated glycosyl donor **3** were accomplished in 81% yield. Interestingly, the N^1 ethyltetrazolyl isomer of **3** was susceptible to hydrolysis in the aqueous $NaHCO_3$ workup that followed the acid-catalyzed acetolysis. As a result, triacetate **3** containing less than 1% of N^1 isomer was obtained in 81% yield with a simple extractive workup. The two epimers of **3** reacted with similar efficiency in the glycosylation conditions when trimethylsilyl trifluoromethanesulfonate (TMSOTf) was used for catalysis.

Substituted purine derivatives were prepared as shown in Scheme 2. Reaction of 2-bromohypoxanthine[12] with L-phenylalaninol afforded guanine **4e** in 74% yield. Acetylation (68%) followed by chlorination with $POCl_3$ (60%) led to 6-chloroguanine **4d** after in situ hydrolysis of the remaining acetyl protecting group. Finally, treatment with ammonia provided 2,6-diaminopurine **4c** in 85% yield.

Coupling of triacetate **3** with purines **4a-f** was carried out with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) as a silylation reagent and TMSOTf as a catalyst. Although most literature procedures employ pre-silylation of the purine base, we found that comparable results were generally obtained with Wright's protocol of direct mixing of the peracetylated riboside and the purine base with BSA and TMSOTf in refluxing MeCN.[4a] Results are summarized in Table 1. Assignment of the 9β structures as shown was based on subsequent conversion of the major product to the target molecule **2**, the structure of which was confirmed by careful NMR studies including NOE and HMBC (heteronuclear multiple bond correlation) experiment. The ratio of major isomer to all minor isomers was determined by HPLC−MS method. In this method, all peaks in the HPLC chromatograms of the quenched reaction mixture or the crude product were identified by molecular weight. The integrals or areas of all peaks of the same molecular weight as the desired isomer were added together and considered the sum of all minor isomers. The ratio was calculated by dividing the area of the desired 9β isomer by the sum of all minor isomers. This ratio determination assumes that all the isomers have the same UV absorption efficiency at the same detection wavelength due to structural similarity.

SCHEME 2

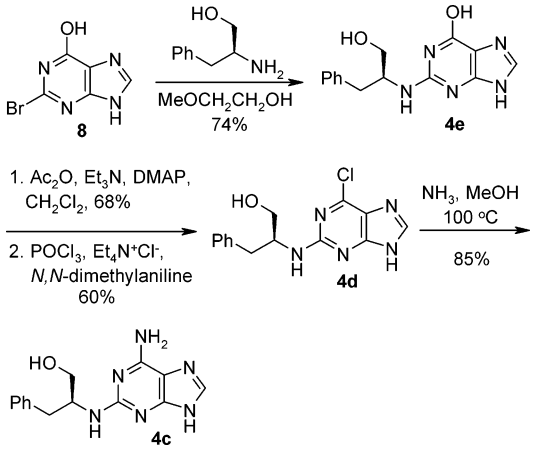
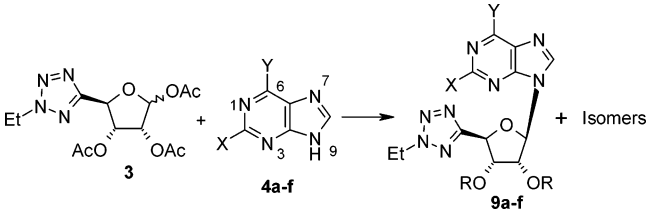


TABLE 1. Glycosylation of Purines 4a−f with Triacetate 3[a]



purine	X	Y	R	9β product	ratio 9β/isomers[b]	9β yield[c] (%)
4a	F	NH_2	Ac	**9a**	29:1	76
4b	Cl	NH_2	H	**9b**	23:1	83
4c	Ph-AlaNH	NH_2	Ac	**9c**	9:1	78
4d	Ph-AlaNH	Cl	Ac	**9d**	93:1	71
4e	Ph-AlaNH	OH	Ac	**9e**	1.8:1	47
4f	Cl	Cl	Ac	**9f**	14:1	78

[a] All reactions were performed with **3** (1.2 equiv), TMSOTf, and BSA in MeCN at reflux. [b] Ratio was determined by HPLC−MS analysis on either the reaction mixture or crude products. [c] **9a** was isolated by crystallization and **9b** by crystallization as diol after deacetylation in the presence of MeOH and K_2CO_3. All others were isolated by chromatography.

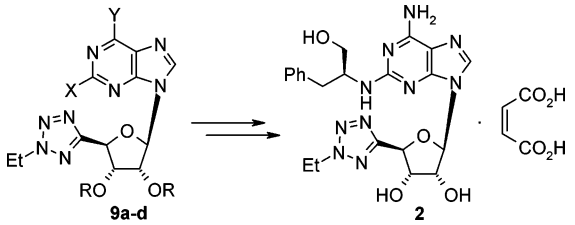
(9) An arabinofuranosyl 2-fluoroadenine was reported to be prepared from a 1-chloroarabinose compound. See: Markovac, A.; Kalamas, R. L.; Lamontagne, M. P. Patent AU/642611, 1993.

(10) Glycosylation of the Na or Hg salt of 6-chloroguanines by 1-chloro- and 1-bromoribofuranoses has been reported, mostly with low yield and poor selectivity. See: (a) Hanna, N. B.; Ramasamy, K.; Robins, R. K.; Revankar, G. R. *J. Heterocycl. Chem.* **1988**, *25*, 1899. (b) Chu, C. K.; Matulic-Adamic, J.; Huang; J.-T.; Chou, T.-C.; Burchenal, J. H.; Fox, J. J.; Watanabe, K. A. *Chem. Pharm. Bull. Med.* **1989**, *37*, 336.

(11) Tetrazolyl compound **5** was prepared from a reaction of the precursor nitrile with NaN_3 in DMF. See: Schmidt, R. R.; Heermann, D.; Jung, K.-H. *Liebigs Ann. Chem.* **1974**, 1856.

(12) Beaman, A. G.; Gerster, J. F.; Robins, R. K. *J. Org. Chem.* **1962**, *27*, 986.

TABLE 2. Conversion of 9a−d to Maleate 2



substrate	X	Y	R	reagents[a] and yield
9a	F	NH_2	Ac	(i) A; (ii) B; (iii) C. 75% for three steps
9b	Cl	NH_2	H	(i) A; 83% from **4b**; (ii) D; (iii) C. 59% for two steps
9c	Ph-AlaNH	NH_2	Ac	(i) A; (ii) C. 62% for two steps
9d	Ph-AlaNH	Cl	Ac	(i) E; (ii) C. 83% for two steps

[a] Key: (A) K_2CO_3, MeOH; (B) L-phenylalaninol, *i*-Pr_2NEt, DMSO, 100 °C; (C) maleic acid, MeOH/EtOH; (D) L-phenylalaninol 105 °C; (E) NH_3, *i*-PrOH, 100 °C.

The ratio thus determined was in agreement with the ^{1}H NMR of the crude products in cases where the ratio was relatively low (**4c**, **4e** and **4f**). No efforts were made to assign structures for the minor isomers which presumably included N^7 and α nucleosides.

Superior selectivity for the desired 9β isomer was obtained with 2-fluoroadenine **4a**[13] (29:1) and 2-chloroadenine **4b**[14] (23:1). The 93:1 selectivity with 6-chloroguanine derivative **4d** was particularly impressive.[15] With the exception of **4e**, all bases provided the desired nucleosides in good isolated yield. In addition to being formed with high stereoselectivity, the product 2-fluoro- and 2-chloroadenosines were found to be highly crystalline, a property which significantly aided isolation and purification in large scale. Although the use of diaminopurine **4c** in the glycosylation reaction represents the most convergent sequence to the desired compound, the selectivity was comparatively low (9:1), as was the selectivity with guanine **4e** (1.8:1).

Vorbruggen has postulated that the initial kinetically formed N^3 isomer rearranges to the thermodynamic N^9 nucleoside via the intermediacy of the N^7 nucleoside.[6] The ratio of N^9 to N^7 products obtained is presumably related to the relative thermodynamic stability of the two isomeric nucleosides. There has been a mechanistic study on isomer distribution in the formation of 6-oxopurines such as **4e**.[4a] In view of the excellent results obtained with use of halogenated purines **4a**, **4b**, and **4d**, studies are ongoing to determine the basis for the observed selectivity among various purine derivatives.

Conversion of **9a**−**d** to **1** was accomplished as shown in Table 2, and the product was isolated as the crystalline maleate salt **2** by simple filtration of the reaction mixture. The assignment of relative structure of **2** was confirmed by NMR analysis which included studies including NOE and HMBC (heteronuclear multiple bond correlation) experiment. The absolute stereochemistry was assigned based on the starting D-ribose structure. Processing of 2-haloadenosines was particularly practical owing to the excellent crystallinity of the nucleoside products of the glycosylation reaction. The chloro displacement of 2-chloroadenosine **9b** was somewhat difficult and required the use of neat phenylalaninol as a melt. However, the displacement with the more reactive 2-fluoro (**9a**) and 6-chloro (**9d**) nucleosides proved facile and provided access to a variety of adenosine analogues with potential for further functionalization in either the 2 or 6 positions of the purine moiety. Separately, guanosine **9e** and ribofuranosyl 2,6-dichloropurine **9f** were converted in several steps to **2** following sequences previously reported in the literature.[4a,5a]

In summary, we have achieved excellent 9β selectivity in glycosylation of 2-haloadenines **4a** and **4b**, as well as N^2-alkyl-6-chloroguanine **4d** by 5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-2,3,4-triyl triacetate **3**. Moreover, these glycosides have been converted to 2-alkylaminoadenosine **1** in the form of its maleate salt **2** by simple halogen displacement at the 2- or 6-position of the purine moiety. We believe that the sequences we described herein provide efficient access to potentially a wide variety of 2-alkylaminoadenosines.

Experimental Section

Representative Procedure for Glycosylation: (2*R*,3*R*,4*R*,-5*R*)-2-(6-Amino-2-fluoro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diyl Diacetate (9a). To a mixture of 65.0 g (190 mmol) of triacetate **3** and 24.1 g (157 mmol) of 2-fluoroadenine **4a** in 372 mL of MeCN was successively added 58.5 mL (238 mmol) of *N*,*O*-bis(trimethylsilyl)-acetamide (BSA) and 34.5 mL (190 mmol) of TMSOTf at ambient temperature. The yellow suspension was heated at reflux and for 5 h. After being cooled to ambient temperature, the reaction was quenched with 390 mL of 10% $KHCO_3$ and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were washed with 400 mL of 10% brine, filtered through a short pad of Celite, and partially concentrated in vacuo to 300 mL. The resultant white slurry was treated with 500 mL of EtOH and concentrated to 350 mL. The product was filtered off and dried in vacuo at 50 °C to afford 51.9 g (76%) of 2-fluoroadenosine **9a** as a white crystalline solid. Before the reaction was quenched, an aliquot was taken and diluted with H_2O−MeCN (1:9). This crude mixture was analyzed by HPLC−MS. The analysis indicated 90% of desired product **9a** along with 3.1% total of five isomeric impurities. The ratio of the desired 9 β product and isomers was thus 29:1. **9a**: mp 208−210 °C dec; $[\alpha]^{20}_D$ −35 (*c* 1.1, MeOH); IR 1763, 1673, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.69 (t, J = 7.5 Hz, 3H), 2.07 (s, 3H), 2.21 (s, 3H), 4.74 (q, J = 7.5 Hz, 2H), 5.58 (s, 1H), 5.78 (dd, J = 4.8, 2.4 Hz, 1H), 5.93 (br s, 2H), 6.16 (dd, J = 6.7, 4.8 Hz, 1H), 6.46 (d, J = 6.7 Hz, 1H), 8.39 (s, 1H). Anal. Calcd for $C_{16}H_{18}FN_9O_5$: C, 44.14; H, 4.17; N, 28.95. Found: C 44.09; H, 4.13; N, 28.78.

Representative Procedure for Preparation of 2: (2*R*,3*R*,-4*S*,5*R*)-2-(6-Amino-2-[[(1*S*)-2-hydroxy-1-(phenylmethyl)-ethyl]amino]-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)-tetrahydro-3,4-furandiol Maleate Salt (2). A mixture of 23.1 g (53.1 mmol) of 2-fluoroadenosine **9a** and 14.7 g (106 mmol) of K_2CO_3 in 215 mL of MeOH was stirred at ambient temperature overnight. The resultant slurry was diluted with 300 mL of EtOAc and poured into 250 mL of water. The aqueous layer was extracted with EtOAc (4 × 200 mL). The combined organic layers were dried with anhydrous Na_2SO_4, concentrated in vacuo, and dried at 60 °C to give 18.5 g (99%) of the 2,3-dihydroxy derivative of 2-fluoroadenosine **9a** as a white solid. A mixture of 18.2 g (52.0 mmol) of this solid and 16.5 g (109 mmol) L-phenylalaninol in 40 mL of DMSO and 120 mL of *i*-Pr_2NEt was heated at 100 °C overnight. The mixture was cooled to 40 °C, diluted with 200 mL of water, and extracted with 20:1 EtOAc−MeOH (4 × 200

(13) Fluoroadenine was purchased from either Allied Signal Specialty Chemicals or Fluorochem Ltd.

(14) Brown, G. B.; Weliky, V. S. *J. Org. Chem.* **1958**, *23*, 125.

(15) Similar selectivity was observed when **4a** and **4b** were glycosylated by β-D-ribofuranose 1,2,3,5-tetraacetate.

mL). The combined organic layers were washed with 200 mL of brine and concentrated in vacuo. The resultant crude product (39 g) was chromatographed on silica gel. Elution with 10% MeOH in CH_2Cl_2 afforded 21.2 g (85%) of **1** as a light yellow solid. A solution of 1.29 g (2.68 mmol) of free base **1** and 311 mg (2.68 mmol) of maleic acid in 16 mL of 95:5 EtOH−MeOH was stirred at ambient temperature for 4 h. The resultant white solid was filtered, washed with 3 mL of EtOH, and dried in vacuo at 60 °C to provide 1.42 g (89%) of maleate salt **2** as a white crystalline solid: mp 169 °C dec; $[\alpha]^{25}_D$ −30.0 (*c* 1.0, MeOH); IR 1705, 1663, 1628, 1508 cm^{-1}; ^1H NMR (MeOH-d_4) δ 1.63 (t, J = 7.3 Hz, 3H), 2.97 (m, 2H), 3.33 (m, 1H), 3.68 (m, 2H),4.38 (m, 1H), 4.68 (m, 1H), 4.72 (q, J = 7.3 Hz, 2H), 4.83 (m, 1H), 5.36 (d, J = 4.5 Hz, 1H), 6.12 (d, J = 5.8 Hz, 1H), 6.30 (s, 2H), 7.13−7.34 (m, 5H), 8.27 (s, 1H). Anal. Calcd for $C_{25}H_{30}N_{10}O_8$: C, 50.16: H, 5.07; N, 23.40. Found: C 50.04; H, 5.07; N, 23.33.

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Supporting Information Available: Detailed experimental procedures and analytical information for compounds **2**, **3**, **4c**−**e**, **6**, and **9a**−**f**, X-ray crystallographic data for **7**, and detailed NMR analysis for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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