Table I. Pharmacological Activity of 1

compound	acetylcholinesterase inhibition: IC_{50},[a] μM	reversal of scopolamine-induced memory impairment: %>CO (best dose, mg/kg sc)[b]	acute toxicity in mice: LD_{50},[c] mg/kg po
1	4.8 ± 1.0	33 (0.63)	136 (113–163)
THA	0.31 ± 0.08	40 (0.63)	39.8 (25.1–63.0)

[a] These values were obtained as described in ref 16, using a rat striatal preparation. They are reported as the mean ± SEM, with $N = 3$ for 1 and $N = 4$ for THA. [b] The procedures for this paradigm are essentially as described in ref 12. In our adaptation, groups of 15 CFW mice were used. A cutoff (CO) was defined for the scopolamine-vehicle group as the value for the animal with the second longest latency time, and results are reported as the percent of animals in the scopolamine-drug group that exhibited latencies greater than the cutoff time (ref 17). The reported dose is that at which the greatest effect was observed. [c] These values were obtained by a modification of the method of Bliss (ref 18). Groups of 10 mice were tested at four doses. The values in parentheses are 95% confidence limits.

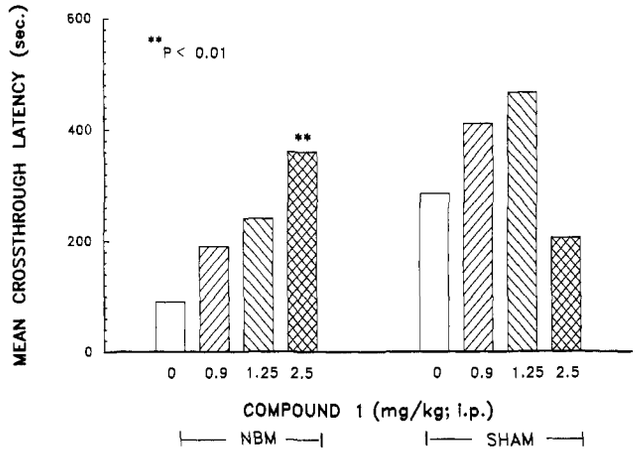

Figure 1. Effect of 1 on the 72-h retention of passive avoidance in NBM lesioned rats. The procedures for this test are described in ref 13. Groups of 10 rats were used per dose; the zero mg/kg dose corresponds to an injection of pure saline. There was a significant impairment of retention test performance in the saline-injected NBM lesioned rats (ANOVA $F = 8.56$, $P < 0.01$, Newman–Keuls test, $P < 0.05$). There was a statistically significant (ANOVA $F = 3.3$, $P < 0.025$) enhancement of retention by HP 029 in both lesioned and sham-operated rats. In the lesioned rats, HP 029 exerted its greatest effect at the 2.5 mg/kg dose (Newman–Keuls test, $P < 0.01$).

THA are summarized in Table I. As an AChE inhibitor, 1 is somewhat less active in vitro than THA and yet is active in the same dose range as THA in reversing scopolamine-induced memory impairment (Table I);[14] it is also active in the NBM model, reversing the retention deficit in the lesioned animals and enhancing learning in the sham-operated animals. At the 2.5 mg/kg dose, the deficit in the lesioned animals was not only reversed but performance was actually improved above the level of normal sham-operated controls (Figure 1).

Compound 1 was further evaluated for acute toxicity in mice (Table I). It can be seen that 1 is significantly less toxic than THA while, as indicated above, 1 and THA are essentially equipotent in reversing scopolamine-induced dementia; this toxicity differential was also observed in longer term studies. Thus, even though 1 is less potent than THA as an AChE inhibitor, it is considerably less toxic and, at the same time, equally effective in an assay that may be predictive of activity in Alzheimer's disease.

In view of these and other results, clinical trials were initiated with 1. In light of the recently reported results with THA, we feel it timely to report that upon completion of phase I with 1, an acceptable dose range for outpatient evaluation has been defined. Neither laboratory nor clinical evidence of drug-induced hepatotoxicity has been reported in 1396 subject days of exposure (normal young and elderly volunteers as well as in AD patients).[15] Further studies to establish the safety and efficacy of 1 in AD are under way.

Registry No. 1, 112964-99-5; 1 (free base), 112964-98-4; 2, 1885-29-6; 3, 504-02-9; 4, 104675-23-2; 5, 104675-26-5; AChE, 9000-81-1.

(14) These data suggest that, in addition to cholinesterase inhibition, there may be other components to the mechanism of action of 1. Further studies aimed at elucidating the mechanism of action are 1 are under way and will be reported at a later date.

(15) (a) Lassman, H. B.; Puri, S. K., unpublished results. (b) Murphy, M. F., unpublished results. Further details of the clinical aspects of this work will be published shortly.

(16) Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.

(17) The high degree of variability (due to season of the year, housing conditions, and handling) found in one-trial passive avoidance paradigms is well known (see: Bammer, G. *Neurosci. Biobehav. Rev.* **1982**, *6*, 274). To control for this fact, individual cutoff (CO) values were determined for each test, compensating for intertest variability. Additionally, it was found that 5–7% of the mice in the scopolamine/vehicle control groups were insensitive to scopolamine at 3 mg/kg, sc. Thus, the CO value was defined as the second highest latency time in the control group to more accurately reflect the 1/15 expected control responders in each test group. Experiments with a variety of standards repeated under a number of environmental conditions led to the development of the empirical positive activity criterion of 3/15 mice with latencies over CO.

(18) Bliss, C. I. *Q. J. Pharm. Pharmacol.* **1938**, *11*, 192.

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Methyl Mercapturate Episulfonium Ion: A Model Reactive Metabolite of Dihaloethanes

Sir:

Dihaloethanes (DHEs), commonly used as soil fumigants, gasoline additives, solvents, and synthetic intermediates,[1] have been found to be carcinogenic in animals.[2] This activity is dependent on both glutathione (GSH) and

(1) Fishbein, L. *J. Toxicol. Environ. Health* **1980**, *6*, 1133.
(2) Storer, R. D.; Conolly, R. B. *Carcinogenesis* **1983**, *4*, 1491.

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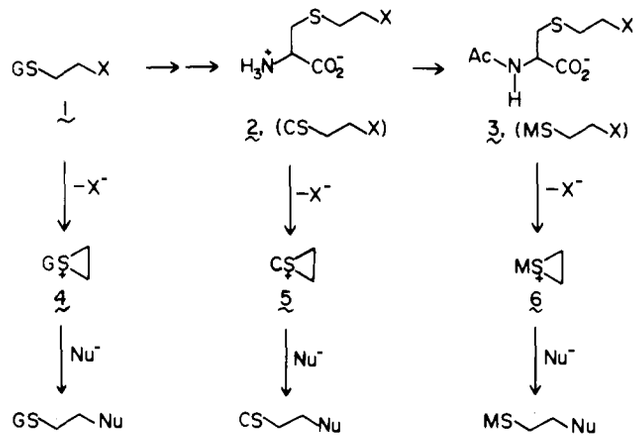
Table I. Selected Proton Chemical Shifts for **9**, **10**, and **7**

compd	CO_2CH_3	NHAc	CH	$CHCH_2S$	SCH_2CH_2	SCH_2CH_2
9[a]	3.81	2.09	4.69	3.06	2.78	3.77
10[b]	3.69	2.12	4.76	3.04	2.94	4.53
7[a]	3.73, 3.78	2.01, 2.04	4.95, 4.65	2.9–3.2	3.7–4.2	

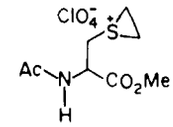
[a] D_2O solvent. [b] Acetone-d_6 solvent.

Scheme I



GSH *S*-transferase.[3-12] It appears that the GSH conjugate **1**, which may be formed in several organs, is converted in the kidney to the cysteine derivative **2**[6] and ultimately to the mercapturic acid derivative **3**.[7] It has been proposed and shown indirectly that the ultimate carcinogens are episulfonium ions (ESIs) **4–6** arising from the corresponding conjugates **1**,[8,9,13] **2**,[10,13] and **3**,[11,12] respectively (Scheme I). Recently the cysteine-based ESI **5**, generated from *S*-(2-haloethyl)-L-cysteine, has been observed by use of NMR spectroscopy.[14] To date, however, there has been no report of such a transient species being isolated, characterized, and studied for solution stability.

The stability of ions such as **4–6** is currently a major topic of investigation. Normally, unsubstituted ESIs are quite unstable and react rapidly with solvent or other nucleophiles in the medium.[15] Both the rate of formation and the degree of reactivity of these ions will bear on the carcinogenic profile of the conjugates, in terms of both alkylation potential and transport to the site of action. We herewith report the first synthesis, isolation, and preliminary study of the hydrolytic stability of an episulfonium ion derivative of *N*-acetylcysteine, the methyl mercapturate episulfonium ion (MMESI, **7**), and some preliminary



7

observations on the behavior of the biologically more relevant nonesterified derivative.

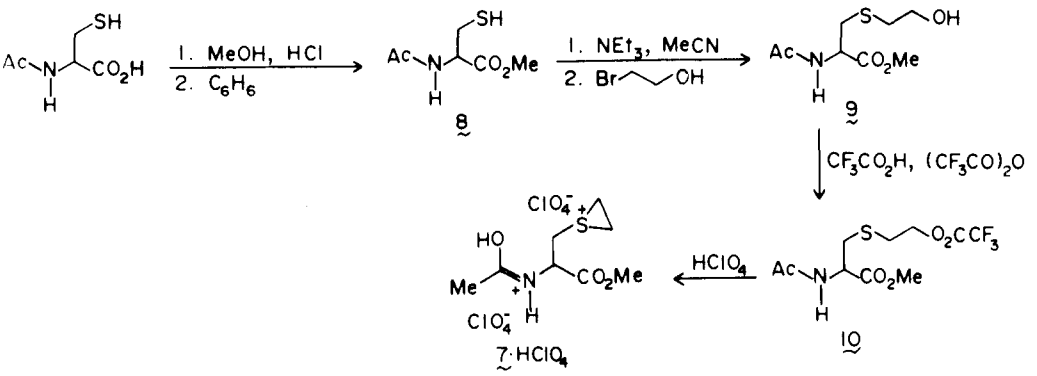
The synthesis of **7** began with mercapturic acid (*N*-acetyl-L-cysteine), which was converted to the methyl ester **8**[16] (Scheme II). This was S-alkylated with 2-bromoethanol and the resulting alcohol **9** was converted to the trifluoroacetate ester **10**. Compounds **8–10** were characterized by both ^1H and ^{13}C NMR spectroscopy (200 and 50 MHz, respectively). The spectra of these compounds were completely consistent with the assigned structures. The ^1H NMR shifts of selected protons are contained in Table I. Treatment of **10** with perchloric acid resulted in the loss of trifluoroacetate[17] plus protonation of the amide to afford **7**·$HClO_4$. This diperchlorate salt, while quite hygroscopic, was an amorphous solid after extensive trituration with anhydrous ether. Remarkably, it showed no evidence of decomposition after 1 week at 23 °C under N_2.

Compound **7**·$HClO_4$ was characterized and investigated by ^1H NMR spectroscopy, first in a nonprotic solvent (acetone-d_6) and then in D_2O solution. To investigate the stability of **7** in water, a small amount of D_2O was added to an NMR tube containing the compound dissolved in acetone-d_6. Interestingly, although the protonated amide reverted to the nonprotonated form (with release of 1 equiv of $HClO_4$), no hydrolysis of the episulfonium ion was observed even 45 min after the addition of D_2O. Subsequent spectra were obtained in D_2O alone at pH <2 (due to the presence of $HClO_4$). Selected NMR data for **7** (in D_2O) are also provided in Table I. The spectra of **7** as both the diperchlorate salt (acetone-d_6) and the monoperchlorate salt (D_2O) showed anomalous signal splitting. In D_2O the methylene protons around the sulfur atom appeared as poorly resolved multiplets, but integrated as expected. The α-methine proton appeared as a doublet of poorly resolved triplets, each integrating for 0.5 H. Each of the two methyl groups appeared as a doublet. The reason for this multiplicity is not yet apparent (vide infra).

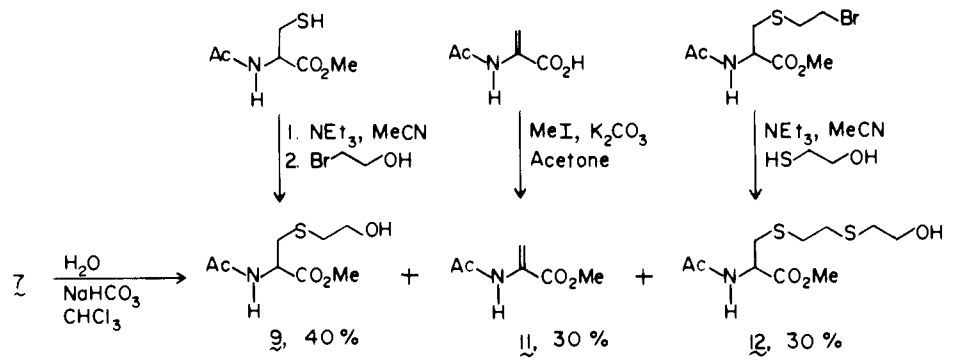
The unusual stability of **7** in D_2O prompted us to study its kinetic behavior, also using ^1H NMR spectroscopy. In unbuffered D_2O at 23 °C (pH ca. 1.5) it exhibited good first-order kinetics through 3 half-lives at a concentration of 0.026 M, with a hydrolysis rate of 0.0315 (±0.0005) h^{-1} and a half-life of 22.0 h. The kinetic profile was readily determined by following either the appearance of the isolated triplet signal of the product (**9**) at δ 2.78 or the disappearance of the episulfonium ring protons signal at δ 3.7–4.2. Agreement between the two methods was quite

(3) Inskeep, P. B.; Guengerich, F. P. *Carcinogenesis* **1984**, *5*, 805.
(4) Storer, R. D.; Conolly, R. B. *Toxicol. Appl. Pharmacol.* **1985**, *77*, 36.
(5) (a) White, R. D.; Gandolfi, A. J.; Bowden, G. T.; Sipes, I. G. *Toxicol. Appl. Pharmacol.* **1983**, *69*, 170. (b) Sipes, I. G.; Wiersma, D. A.; Armstrong, D. J. *Adv. Exp. Biol. Med.* **1986**, *197*, 457.
(6) Anders, M. W.; Lash, L. H.; Elfarra, A. A. *Adv. Exp. Biol. Med.* **1986**, *197*, 443.
(7) van Bladeren, P. J.; Breimer, D. D.; van Huijgevoort, J. A. T. C. M.; Vermeulen, N. P. E.; van der Gen, A. *Biochem. Pharmacol.* **1981**, *30*, 2499.
(8) van Bladeren, P. J.; van der Gen, A.; Mohn, G. R. *Biochem. Pharmacol.* **1979**, *28*, 2521.
(9) Shih, T.-W.; Hill, D. L. *Res. Commun. Chem. Pathol. Pharmacol.* **1981**, *33*, 449.
(10) Webb, W. W.; Elfarra, A. A.; Webster, K. D.; Thom, R. E.; Anders, M. W. *Biochemistry* **1987**, *26*, 3017.
(11) Rannug, U.; Beije, B. *Chem.-Biol. Interact.* **1979**, *24*, 265.
(12) van Bladeren, P. J.; Breimer, D. D.; Rotteveel-Smijs, G. M. T.; deJong, R. A. W.; Buijs, W.; van der Gen, A.; Mohn, G. R. *Biochem. Pharmacol.* **1980**, *29*, 2975.
(13) Foureman, G. L.; Reed, D. J. *Biochemistry*, **1987**, *26*, 2028.
(14) Dohn, D. R.; Casida, J. R. *Bioorg. Chem.* **1987**, *15*, 115.
(15) Smit, W. A.; Zefirov, N. S.; Bodrikov, I. V.; Krimer, M. Z. *Acc. Chem. Res.* **1979**, *12*, 282.
(16) van Bladeren, P. J.; Buys, W.; Breimer, D. D.; van der Gen, A. *Eur. J. Med. Chem.* **1980**, *15*, 495.
(17) Ohishi, J.; Tsuneoka, K.; Ikegami, S.; Akaboshi, S. *J. Org. Chem.* **1978**, *43*, 4013.

Scheme II



Scheme III



consistent and within the stated experimental error. In the latter method the integration measurements showed a change from seven to five protons, due to the presence of the methyl ester protons and the methylene protons α to the hydroxyl group of **9**. After several days, hydroxy ester **9** and the corresponding carboxylic acid (which also showed a triplet at δ 2.78) were the only compounds present. Since the solutions were unbuffered, we also noted a dependence of the rate on the pH of the solution, with a lower pH (more concentrated ion) running slightly slower.

Preliminary results with the ESI **6** showed that it was also stable in an acidic aqueous medium and appeared to behave in a manner analogous to **7**. More detailed kinetic and biological studies of both of these compounds are under way and will be reported in a future paper.

To compare the electrophilic reactivity of **7** to that of other known ESIs, it was exposed to two common nucleophiles, methanol and tetramethylammonium chloride. The results were as expected: formation of the respective methyl ether and β-chloro derivatives. The structure of the chloro derivative was unequivocally established by comparison with an authentic sample, obtained from alkylation of the methyl ester of mercapturic acid with 1-bromo-2-chloroethane. The structure of the methyl ether derivative was established by ^1H and ^{13}C NMR spectroscopy. The reactivity of **7** toward these nucleophiles is comparable to that seen with other ESIs.[18]

We expected that under neutral or slightly basic aqueous conditions **7** would be rapidly and quantitatively converted to alcohol **9**. To our surprise, this was not the case. When placed in 0.9 M sodium bicarbonate solution (pH 8), **7** did react very quickly but yielded only approximately 40% of the expected alcohol **9** (Scheme III). The remainder was an almost equal mixture of two other products, **11** and **12**. Each of the three products was separated by flash chromatography and identified unequivocally by comparison to an independently synthesized authentic sample by use of ^1H and ^{13}C NMR spectroscopy. Alcohol **9** was already on hand as a synthetic intermediate for **7**, while **11** was synthesized by esterification of acetamidoacrylic acid (Aldrich). The unusual product **12** was prepared by bromination of **9**, followed by reaction with mercaptoethanol.

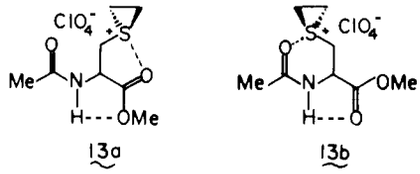
Formation of **11** from **7** may be rationalized as follows. Unlike the other ESIs that have been isolated to date, **7** has an acidic proton β to the episulfonium group. Since the charged sulfur is a fairly good leaving group and is in a labile position, base-catalyzed elimination of thiirane may compete with nucleophilic attack on the ring. Formation of **12** may be rationalized in several ways, one of which involves the reaction of the thiirane released when **11** is formed. Thiirane is a fair nucleophile[18] and may react with another molecule of **7** to form a second episulfonium ion, which is then hydrolyzed in the usual way. A similar elimination pathway is thought to occur in the metabolism of 1,4-dihalobutanes.[19]

The anomalous splitting of the proton signals in the NMR spectrum of **7** are being investigated further. This splitting is associated only with **7** and not with either the immediate precursor (**10**) or the hydrolysis product (**9**). On first look, the signals of the methine proton and the two methyl groups appear to correspond to two separate (and presumably stable) solution conformations of **7**. If such a conformational heterogeneity is present, it may be due to the episulfonium group being stabilized by interactions with the neighboring carbonyl oxygens. To determine the feasibility of such interactions, we investigated the compound using a molecular graphics package, PCMODEL.[20] Two conformations, **13a** and **13b**, could be generated in

(18) Owsley, D. C.; Helmkamp, G. K.; Spurlock, S. N. *J. Am. Chem. Soc.* **1969**, *91*, 3606.

(19) (a) Marchand, D. H.; Abdel-Monem, M. M. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 360. (b) Onkenhout, W.; van Loon, W. M. G. M.; Buijs, W.; van der Gen, A.; Vermuelen, N. P. E. *Drug. Metab. Disp.* **1986**, *14*, 608, and references cited therein.

(20) Henkel, J. G.; Clarke, F. H. *Molecular Graphics on the IBM PC*; Academic: Orlando, FL, 1986.



13a 13b

which the positive sulfur and carbonyl oxygen were within 2.3 Å of one another, with neither having any apparent severe steric strain. Such interactions, if present, would be expected to decrease the effective positive charge on the sulfur, stabilizing the episulfonium ring and rendering it less susceptible to nucleophilic attack. The existence of such an unexpected conformational distribution is presently being investigated.

Several conclusions can be drawn from this preliminary study. First, **7** has a half-life on the order of hours in strongly acidic water at ambient temperature. This is in contrast to simple aliphatic ESIs which often cannot even be isolated at room temperature. Second, ESIs with relatively acidic β protons are likely to undergo elimination as well as nucleophilic addition under physiological conditions. Since **4** and **6** also have the necessary structural requirements to undergo elimination, this decomposition route may be relevant for them as well. Such a route could play a role in the toxicological profile of the DHEs. Third, if significant intramolecular stabilization is present in **7**, as indicated (but not proven) by the NMR and modeling data, it would support increased stability for the putative ultimate carcinogens **4** and **6**, which also have suitably positioned carbonyls. The cysteine-based ESI **5** is predicted to be less stable by this argument. This hypothesis is presently being tested and further studies on these and compounds based on the parent molecules (GSH and MA) will be reported in a subsequent paper.

Acknowledgment. We thank Boehringer Ingelheim, Ltd., Ridgefield, CT, for support of this work in the form of a fellowship to G.S.A., and Dr. James B. Mangold for stimulating discussions.

Registry No. **7**, 114301-30-3; **7**·$HClO_4$, 114301-33-6; **8**, 7652-46-2; **9**, 77109-48-9; **10**, 114301-31-4; **11**, 35356-70-8; **12**, 114301-32-5; $BrCH_2CH_2OH$, 540-51-2; $HSCH_2CH_2OH$, 60-24-2.

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N^6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine and Its Uronamide Derivatives. Novel Adenosine Agonists with Both High Affinity and High Selectivity for the Adenosine A_2 Receptor

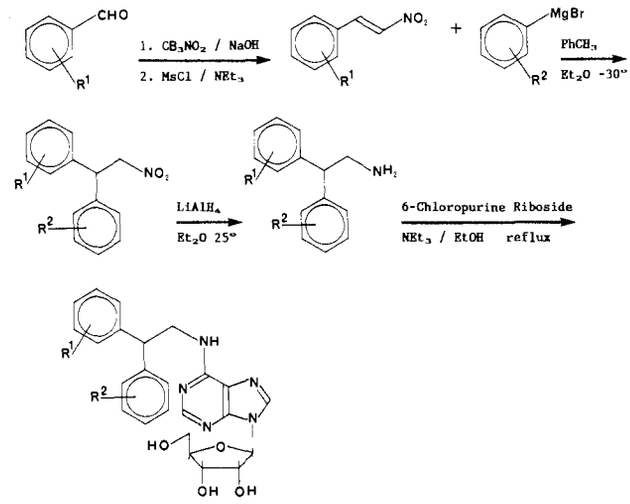
Sir:

Extracellular adenosine acts as a local hormone, operating through two major subclasses of membrane-bound adenosine receptors, called A_1 and A_2, which are distinguished by their structure–activity relationships.[1-3] Relative affinities of compounds for these adenosine receptor subtypes can be determined by using specific A_1 and A_2 receptor binding assays.[4] Many potent and selective A_1 agonists have been found, typified by *R*-PIA (**1**)[5] and CPA (**2**)[6] (Table I and Chart I). However, progress toward potent and selective A_2 agonists has been much slower. For instance, NECA (**3**),[7] although very potent at A_2 receptors,[8] is slightly A_1 selective in the A_1 and A_2 binding assays.[4] Among the few known A_2-selective agonists are 2-(phenylamino)adenosine (CV-1808, **4**)[8] and N^6-(1-naphthalenylmethyl)adenosine (**5**).[9-11] Although the former compound has the greatest A_2 selectivity yet reported, its selectivity is only 5-fold and its binding affinity is 1 order of magnitude lower than that of NECA.[4] In this communication we wish to report the discovery of a series of agonists with 2–4-fold stronger A_2 affinity than NECA, which show up to a 40-fold selectivity for the A_2 receptor.

Recently we reported[12] that N^6-(2,2-diphenylethyl)-adenosine (CI-936, **6**) is a moderately potent A_2 agonist with 25 nM A_2 affinity, a nearly balanced receptor binding profile, and an excellent profile in behavioral tests predictive of antipsychotic-like activity. Simply bridging the phenyl rings, to give N^6-(9-fluorenylmethyl)adenosine (**7**),[11] markedly increases the affinity at the A_2 receptor, to give a balanced agonist with 5 nM affinities at both receptors. Results such as these convinced us that a detailed examination of this series might be fruitful in a search for potent, A_2-selective adenosine agonists.

Several different conceptual approaches to the modification of the 2,2-diphenylethyl side chain of **6** were examined. As alluded to above, bridging of the two phenyl

Scheme I. Synthesis of N^6-(2,2-Diarylethyl)adenosines



(1) Hamprecht, B.; van Calker, D. *Trends Pharmacol. Sci.* **1985**, *6*, 153.
(2) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. In *Topics and Perspectives in Adenosine Research*; Gerlach, E., Becker, B., Eds; Springer-Verlag, Berlin, 1987; p 59.
(3) Ukena, D.; Olsson, R. A.; Daly, J. W. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 365.
(4) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
(5) Vapaatalo, H.; Onken, D.; Neuvonen, P.; Westermann, E. *Arzneim.-Forsch.* **1975**, *25*, 407.
(6) Moos, W. H.; Szotek, D. L.; Bruns, R. F. *J. Med. Chem.* **1985**, *28*, 1383.
(7) Prasad, R. N.; Bariana, D. S.; Fung, A.; Savic, M.; Tietje, K.; Stein, H. H.; Brondyk, H.; Egan, R. S. *J. Med. Chem.* **1980**, *23*, 313.
(8) Kawazoe, K.; Matsumoto, N.; Tanabe, N.; Fujiwara, S.; Yanagimoto, M.; Hirata, M.; Kikuchi, K. *Arzneim.-Forsch.* **1980**, *30*, 1083.
(9) Jahn, W. *Arzneim.-Forsch.* **1969**, *19*, 701.
(10) Kusachi, S.; Thompson, R. D.; Yamada, N.; Daly, D. T.; Olsson, R. A. *J. Med. Chem.* **1986**, *29*, 1628.
(11) Trivedi, B. K.; Bristol, J. A.; Bruns, R. F.; Haleen, S. J.; Steffen, R. P. *J. Med. Chem.* **1988**, *31*, 271.
(12) Bridges, A. J.; Moos, W. H.; Szotek, D. L.; Trivedi, B. K.; Bristol, J. A.; Heffner, T. G.; Bruns, R. F.; Downs, D. A. *J. Med. Chem.* **1987**, *30*, 1709.

0022-2623/88/1831-1282$01.50/0