Effects of Deuteration on Locomotor Activity of Amphetamine[1]

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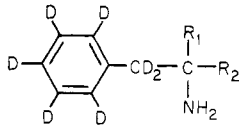
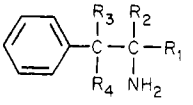
The synthesis of fully deuterated amphetamine (phenyl-2-aminopropane-d_{11}) in which 11 deuterium atoms are bonded to carbons and two other highly deuterated analogues is described. Their toxicities and in vivo spontaneous locomotor activities in mice were examined and compared with that of the parent protioamphetamine. A significant reduction in toxicities and a decrease in spontaneous locomotor activity were observed for these highly enriched deuterated analogues, as compared to protioamphetamine.

Amphetamine is a unique drug with respect to the simplicity of its structure and the multiplicity of its biochemical and pharmacological effects. This has made the molecule an ideal target for extensive molecular modification in order to accentuate some of its effects and/or abolish others. For example, Horn and Snyder[2] synthesized *cis*- and *trans*-2-phenylcyclopropylamines as rigid analogues of amphetamine to examine the steric requirements for its inhibition of neuronal uptake of norepinephrine. Tessel et al.[3] studied the structure–activity relationships of meta-substituted *N*-ethylamphetamines and their spontaneous locomotor activity.

As part of the synthetic design to study the effect of deuteration on drug biotransformations and pharmacological actions, we synthesized the fully and highly enriched deuterated amphetamines (1–3) and examined their in vivo pharmacological effect(s) on spontaneous locomotor activity. To our knowledge, the in vivo effect of deuteration on amphetamine actions has not been reported.

Isotopically altered drugs have shown widely divergent pharmacological effects. Elison et al.[4] reported a reduced analgesic potency and a 24% increase in the basicity of morphine deuterated in the *N*-methyl group. Foreman and co-workers[5] found an isotope effect of 1.9 in the in vitro metabolism of α-deuterated amphetamine 4 with rabbit liver homogenate. This suggests that the deuterioamphetamine is metabolized at a slower rate than its protio form. Deuterium isotope effects in man have been reported for the metabolism of *N*-alkyl-deuterated and α-deuterated amphetamines.[6] A 12% increase in the basicity and a decreased lipid solubility were observed. These observations suggested that further deuteration of the amphetamine molecule such as compounds 1–3 might

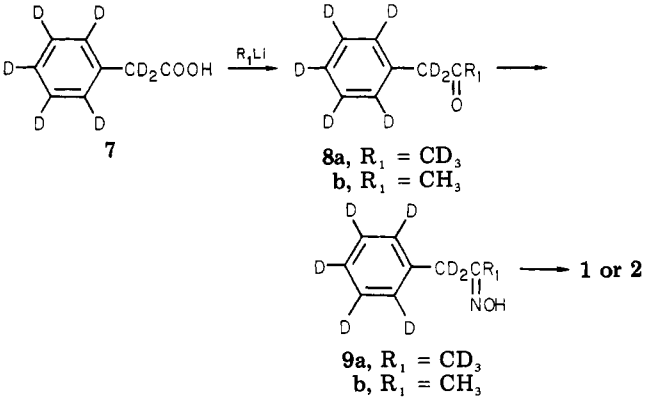




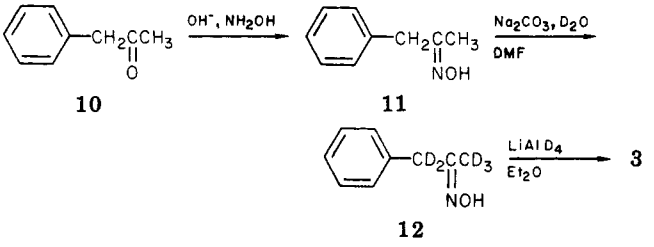
1, $R_1 = CD_3$; $R_2 = D$
2, $R_1 = CH_3$; $R_2 = H$
3, $R_1 = CD_3$; $R_2 = R_3 = R_4 = D$
4, $R_1 = CH_3$; $R_2 = D$; $R_3 = R_4 = H$
5, $R_1 = CH_3$; $R_2 = R_3 = H$; $R_4 = D$
6, $R_1 = CH_3$; $R_2 = R_3 = R_4 = D$

lead to a more pronounced alteration of the physicochemical properties of the drug and, hence, its in vivo pharmacological response. In this paper, we report the synthesis and the in vivo spontaneous locomotor activity of these highly deuterated amphetamines. These activities were compared to other deuterated amphetamines (i.e., 4–6) which have been synthesized previously in this laboratory.[7]

Scheme I



Scheme II



Chemistry. The synthetic routes to the desired compounds are outlined in Schemes I and II. All compounds are racemic where this is possible.

Most of the methods that are described in the literature concerning the synthesis of amphetamine and its derivatives employ the readily available phenylacetone (**10**) as the starting material. To effect the synthesis of fully deuterated amphetamine 1, it was necessary to prepare the unreported phenyl-2-propanone-d_{10} (**8a**). To do this, the method of Tegner[8] for the preparation of nondeuterated phenylacetone was employed. The action of lithium metal on methyl-d_3 iodide in anhydrous ether furnished methyl-d_3-lithium which upon coupling with phenylacetic-d_7 acid (**7**) in anhydrous ether afforded the fully deuterated phenylacetone **8a** in good yield. Treatment of this ketone with hydroxylamine hydrochloride in sodium deuterioxide–deuterium oxide medium yielded phenyl-

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Table I. Comparison of Mean Peak Time, Locomotor Activity at Peak Time, Area under the Time-Response Curve (AUC), and LD_{50} of Protioamphetamine and Its Deuterated Analogues

Compd	Mean peak time, min	Locomotor act. at peak time, (net counts)$^{1/2}$ ± SE	AUC, cm^2	LD_{50},[a] mg/kg	Potency ratio[a]
Protioamphetamine (S)	70	93 ± 0.82	350	123.4 (113.3-134.5)	
Amphetamine-d_{11} (1)	120	81 ± 1.08	350	148.7 (134.0-165.1)	1.20 (1.03-1.39)[b]
Amphetamine-d_7 (2)	80	85 ± 1.70	349	139.5 (125.6-154.8)	1.13 (0.97-1.31)
Amphetamine-d_6 (3)	100	85 ± 1.70	367	142.7 (129.8-157.0)	1.16 (1.00-1.35)[b]
Amphetamine-d_1 (4)	100	92 ± 1.70	370		
Amphetamine-d_1 (5)	75	93 ± 1.24	355		
Amphetamine-d_3 (6)	105	93 ± 1.24	374		

[a] The values in parentheses represent 95% confidence limits. [b] $p \leq 0.05$ vs. protioamphetamine.

2-propanone-d_{10} oxime (**9a**). Lithium aluminum deuteride reduction of the oxime in anhydrous ether furnished the desired fully deuterated *dl*-phenyl-2-aminopropane-d_{11} (**1**), as depicted in Scheme I.

The *dl*-phenyl-2-aminopropane-d_7 (**2**) was prepared in a similar manner as described previously. Phenylacetic-d_7 acid (**7**) was coupled with the readily available methyllithium in anhydrous ether to furnish phenyl-2-propanone-d_7 (**8b**). Conversion of this ketone to its oxime **9b**, followed by lithium aluminum hydride reduction of the resulting oxime, furnished the desired *dl*-phenyl-2-aminopropane-d_7 (**2**).

The preparation of *dl*-phenyl-2-aminopropane-d_6 (**3**) was accomplished by the lithium aluminum deuteride reduction of phenyl-2-propanone-d_5 oxime (**12**) in ether. The oxime **12** was readily prepared by deuterium exchange of the nondeuterated oxime **11** in deuterium oxide, dimethylformamide, and anhydrous sodium carbonate mixture as shown in Scheme II.

A small quantity (approximately 50 mg) of each pure deuterated amphetamine sulfate (**1**–**3**) was converted to the free amine and to the corresponding trifluoroacetamide (TFA) derivatives[9] for the determination of the chemical and isotopic purity. Thus, the presence of aziridine by-product(s)[10-12] in the purified deuterated amphetamines can be eliminated on the basis of TLC and GLPC analysis of the free amines as well as their TFA derivatives. The isotopic purity of the TFA derivatives was estimated according to the method of Lindeke and Cho.[9,13] Thus TFA derivatives of deuterated amphetamines **1** and **3** were estimated by mass spectral analyses to be 99.7 and 99.2%, respectively, by comparison of the base peak (*m/e* 144, $CF_3CONHCDCD_3^+$) for the deuterium enriched compound with the peak at *m/e* 140 for the corresponding natural fragment. The isotopic purity of deuterated amphetamine **2** was estimated to be 98.7% by comparing the mass spectrometer response at *m/e* 124 (65% of the base peak at *m/e* 140) with the peak at *m/e* 118 ($PhCHCHCH_3^+$) of the corresponding natural fragment. Therefore, the minimum isotopic purities of the highly enriched deuterated amphetamine salts prepared in this investigation are greater than 98%.

It is interesting to note that the deuterium exchange of an oxime has not been reported and, hence, it represents a new and easy approach to the synthesis of amphetamine with complete deuteration at the side chain. For example, phenyl-2-propanone-d_{10} oxime (**9a**) can be prepared quantitatively from **9b** by the deuterium exchange procedure. This method provides a practical alternative to the synthesis of *dl*-phenyl-2-aminopropane-d_{11} (**1**) described in Scheme I. The tedious in situ preparation of methyl-d_3-lithium is avoided and the purity of the product of the coupling reaction between phenylacetic-d_7 acid and the readily available methyllithium is improved.

Analogues **5** and **6** were synthesized from benzaldehyde-d_1 and will be reported elsewhere.[7] α-Deuterated amphetamine **4** was synthesized according to the procedure of Foreman et al.[5]

Results and Discussion

As shown in Table I, the LD_{50} values of all deuterio analogues tested were increased. Even though the 95% confidence limits overlapped, potency ratio calculations indicated statistically significant increases for the fully deuterated analogue **1**. Thus, a 20% increase in the LD_{50} for the fully deuterated analogue **1** ($p < 0.05$) was noted, whereas 13 and 16% increases were observed for analogues **2** ($p > 0.05$) and **3** ($p = 0.05$), respectively. The areas under the time–response curve (AUC) as measured by the trapezoidal method for all the deuterated amphetamine analogues were similar to that of the protio form. This indicates that there was no difference in total activity. In addition, there was no significant difference between the descending slopes of each deuterio analogue and the protioamphetamine, indicating a similar mechanism of elimination.

A decrease in spontaneous locomotor activity, as evidenced by a lower activity count, was observed for the highly enriched deuterated analogues **1** ($p < 0.001$), **2** ($p < 0.01$), and **3** ($p < 0.01$) as compared to the protioamphetamine. These observations correspond well with the observed difference in acute LD_{50} values listed in Table I. Little or no significant differences were noted for other deuterated analogues (i.e., **4**–**6**). However, a noticeable difference in mean peak time (a shift of 30–50 min) was observed for the deuterated analogues **1**, **3**, **4**, and **6**. Since all these analogues contained a deuterium atom substituted at the α position of the amphetamine molecule, one may conclude that the deuterium substitution at this position is of importance with regard to the onset of action and peak time. Vree et al.[6] have reported a 12% increase in the basicity and a decrease in the lipid solubility of amphetamine upon deuteration of the α-hydrogen. It is apparent, then, that the basicity of a drug may be proportional to the number of deuterium atoms substituted close to this basic center. Furthermore, since the unionized amine base preferentially enters the brain, enhancement of basicity in deuterated analogues **1**, **3**, **4**, and **6**, as noted above, may explain the differences in the rate and amount of the drug reaching the general circulation and also the brain. On the other hand, no differences in total activity were observed for all deuterated amphetamine analogues as measured by total areas under the curve.

It is interesting to note that amphetamine-d_1 (**5**) is the only deuterated analogue tested that did not differ from

the protioamphetamine both in mean peak time and peak intensity. This suggests that any deuterium substitution at the benzylic portion of the amphetamine molecule does not affect its spontaneous locomotor activity.

A comparison of the fully deuterated amphetamine analogue **1** with analogues **2** and **3** further reveals that the complete deuteration of the aromatic nucleus only affects the potency of the drug but not its onset of action as measured by mean peak time. On the other hand, deuteration of the methyl group, as seen in analogue **3**, might also be responsible for the significant reduction in peak intensity and/or toxicity.

The data obtained in the present study do not permit one to conclude any possible biochemical implications of deuteration on amphetamine actions. However, in vitro studies of these highly enriched deuterated amphetamines on neuronal uptake and/or release of norepinephrine on monoamine oxidase activity, as well as on physicochemical and pharmacokinetic properties of these deuterated analogues, are in progress and will be the subject of future communications.

Experimental Section

Phenylacetic-d_7 acid, methyl-d_3 iodide and lithium aluminum deuteride ($LiAlD_4$) were purchased from Merck Co., Inc. All melting points were determined with a Mel-Temp apparatus and are uncorrected. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill. The IR spectra were recorded neat on a Perkin-Elmer 257 grating infrared spectrophotometer. NMR spectra were obtained in $CDCl_3$ on a Varian T-60A spectrometer. Chemical shifts are reported as parts per million relative to Me_4Si as an internal reference. Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6D MS. TLC was performed on Eastman chromagram silica gel sheets with fluorescent indicator, using $CHCl_3$–MeOH (95:5) and $CHCl_3$–MeOH (4:1) as developing solvents. GLPC analyses were obtained on a Packard Model 7300 chromatograph with a 4 ft × 0.125 in. o.d. glass column packed with Chromosorb 101, 80–100 mesh, using nitrogen as a carrier gas (62 mL/min). Chromatographic conditions were column oven 230 °C, injection port 250 °C, and detector block 250 °C. Where analyses are indicated by the symbols of the elements, the analytical results obtained for these elements were within 0.4% of the theoretical values.

Phenyl-2-propanone-d_{10} Oxime (9a). A solution of phenylacetic-d_7 acid (**7**, 2.0 g, 14 mmol) in anhydrous ether (35 mL) was placed in a clean and dry three-necked round-bottom flask equipped with a magnetic stirrer, a dropping funnel, a gas inlet tube, and a reflux condenser with a drying tube on top. Under nitrogen atmosphere and with rapid stirring an ethereal solution of methyl-d_3-lithium [70 mL, freshly prepared from methyl-d_3 iodide (5.3 g, 36.5 mmol) and lithium metal (0.6 g, 86.5 mmol) in anhydrous ether] was added dropwise at such a rate sufficient to maintain gentle reflux. After the addition of all the methyl-d_3-lithium solution, the mixture was refluxed on a steam bath for 15 min. The solution was allowed to cool to room temperature and 15 mL of water was added slowly. The aqueous layer was removed, the ethereal layer washed three times with water and dried ($MgSO_4$), and the solvent removed under reduced pressure yielding 1.5 g (75%) of **phenyl-2-propanone-d_{10} (8a)** as a colorless oil: IR (neat) 1690 (C=O), 2250 cm^{-1} (CD); mass spectrum m/e 144 (M^+), 98 ($C_6D_5CD_2^+$), 46 (CD_3CO^+). A mixture of phenyl-2-propanone-d_{10} (**8a**, 1.5 g, 10.4 mmol) and hydroxylamine hydrochloride (0.88 g, 12.7 mmol) in D_2O (3.2 mL) was warmed in a water bath maintained at 40 °C. With vigorous stirring, 2.4 mL of 10 N NaOD in D_2O was added dropwise. After the addition of all of the base, the mixture was stirred at 40 °C for 20 min. At this time, an additional amount of hydroxylamine hydrochloride (0.88 g) and 10 N NaOD (2.4 mL) was added in the same manner to ensure completion of the reaction. The mixture was then neutralized by the slow addition of glacial acetic acid (1.5 mL) and was extracted with ether (2 × 100 mL). The combined ether extract was washed with a saturated solution of calcium chloride, dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo affording 1.26 g of **9a** (84% yield) as a colorless oil which thickened on standing: IR (neat) 3250 (OH), 2300 (CD), and 1640 cm^{-1} (C=N); NMR δ 10.3 (br, 1 H, NOH); mass spectrum m/e 159 (M^+), 140 (M^+ – HOD), 98 ($C_6H_5CD_2^+$). This product was used for subsequent reaction without further purification.

Phenyl-2-propanone-d_7 Oxime (9b). This compound was prepared analogously to **9a** beginning with phenylacetic-d_7 acid (1.9 g, 13.4 mmol) and methyllithium (18 mL, 32 mmol). **Phenyl-2-propanone-d_7 (8b)** was obtained in 83% yield (1.55 g): IR (neat) 1690 (C=O), 2250 cm^{-1} (CD); NMR δ 1.87 [s, 3 H, $CH_3C(=O)$–]; mass spectrum m/e 141 (M^+), 98 ($C_6H_5CD_2^+$), 43 (CH_3CO^+).

The desired oxime **9b** was then readily prepared in a similar manner as described above from **8b** (1.4 g, 9.7 mmol), hydroxylamine (1.64 g, 24 mmol), and 10 N NaOH (4.4 mL) in D_2O (3 mL) in 84% yield (1.18 g) and as a viscous oil: IR (neat) 3250 (OH), 2300 (CD), and 1640 cm^{-1} (C=N); NMR δ 1.77 (s, 3 H, CH_3), 10.3 (br, 1 H, NOH); mass spectrum m/e 156 (M^+), 137 (M^+ – HOD), 98 ($C_6H_5CD_2^+$). This product was used for subsequent reaction without further purification.

Phenyl-2-aminopropane-d_{11} (1). A solution of the oxime **9a** (1.26 g, 7.9 mmol) in anhydrous ether (60 mL) was added through a dropping funnel to a vigorously stirred suspension of $LiAlD_4$ (0.44 g, 10.5 mmol) in anhydrous ether (15 mL). The mixture was refluxed for 7 h. After cooling, the excess hydride was decomposed by slow addition of water (1.0 mL), followed by the hydrolysis of the LiAl complex with 10% NaOH solution (3 mL) and stirring at room temperature for 30 min. The suspension was then filtered and the precipitate washed with ether (50 mL). The combined filtrate and washings were dried ($MgSO_4$) and filtered, and the solvent was removed in vacuo, affording 0.63 g (50%) of liquid **1**. The base was converted to the sulfate by the addition of an exactly equivalent amount of ethereal sulfuric acid to a cool ethereal solution of the base. The salt was recrystallized from dilute aqueous ethanol yielding 0.7 g: mp 310 °C dec (lit.[14] mp 310–320 °C dec for the nondeuterated salt); IR (KBr) 3360 and 3270 (NH_2), 2270 cm^{-1} (CD); NMR, no signals; CI mass spectrum m/e 147 ($M^+ + 1$). Anal. ($C_{18}H_6D_{22}N_2O_4S$) C, H, N; D: calcd, 11.35; found, 10.46.

Phenyl-2-aminopropane-d_7 (2). The oxime **9b** (1.1 g, 6.9 mmol) was reduced in a similar manner with $LiAlH_4$ (1.0 g, 26.3 mmol) in anhydrous ether. The yield was 1.1 g of the free amine **2** and 0.88 g of the purified sulfate salt: mp 310 °C dec; IR (KBr) 3360 and 3270 (NH_2), 2270 cm^{-1} (CD); NMR δ 1.10 (d, 3 H, CH_3), 1.7 (s, 2 H, NH_2), and 3.17 (q, 1 H, $-CHCH_3$); CI mass spectrum m/e 143 ($M^+ + 1$). Anal. ($C_{18}H_{14}D_{14}N_2O_4S$) C, H, D, N.

Phenyl-2-propanone-d_5 Oxime (12). A mixture of phenyl-2-propanone oxime (**11**, 3.0 g, 20 mmol), dimethylformamide (DMF, 10 mL), D_2O (10 mL), and anhydrous sodium carbonate (2 g) was refluxed under gentle stirring for 24 h. The mixture was then allowed to cool to room temperature, acidified with 10% aqueous HCl, and extracted with ether (3 × 50 mL). The combined extracts were washed with H_2O (5 × 60 mL), dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure. The deuterium exchange reaction was repeated for an additional 24 h with fresh DMF, D_2O, and anhydrous Na_2CO_3, as before, to ensure complete exchange. Similar workup yielded 2.0 g (97%) of the desired oxime **12**: IR (neat) 3250 (NOH), 2270 (CD), 1640 cm^{-1} (C=N); NMR δ 7.30 (s, 5 H, ArH), 10.3 (s, 1 H, NOH); mass spectrum m/e 154 (M^+), 135 (M^+ – HOD), 93 ($C_6D_5CD_2^+$). Anal. ($C_9H_6D_5NO$) C, H, D, N.

Phenyl-2-aminopropane-d_6 (3). This compound was prepared in a similar manner described for **1** from phenyl-2-propanone-d_5 oxime (**12**, 2.0 g, 13 mmol) and $LiAlD_4$ (0.62 g, 15 mmol) in anhydrous ether (20 mL). After workup in the usual manner, 1.6 g of the free amine was obtained and converted immediately to its sulfate salt which was recrystallized from dilute aqueous ethanol yielding 0.7 g of pure **3**: mp 310 °C dec; IR 3360 and 3270 (NH_2), 2270 cm^{-1} (CD); NMR δ 7.30 (s, 5 H, ArH), 1.33 (s, 2, NH_2); CI mass spectrum m/e 142 ($M^+ + 1$). Anal. ($C_{18}H_{16}D_{12}N_2O_4S$) C, H, D, N.

Trifluoroacetyl Derivatives of *dl*-Phenyl-2-aminopropanes. Approximately 50 mg of pure sulfate salts of amphetamine-d_0, amphetamine-d_{11} (**1**), amphetamine-d_7 (**2**), and amphetamine-d_6 (**3**) was dissolved in H_2O (5 mL) separately and neutralized with solid sodium carbonate. The free amines lib-

erated were extracted into dichloromethane (2 × 10 mL). The combined dichloromethane extracts were dried (K_2CO_3) and TLC analyses showed only a single spot in all cases. The GLPC analyses of these amines showed single peak for amphetamine-d_0 (7.8 min), amphetamine-d_{11} (7.3 min), amphetamine-d_7 (7.6 min), and amphetamine-d_6 (7.3 min). Trifluoroacetic anhydride (0.5 mL) was added to each of the dried dichloromethane solutions and the mixture was left at room temperature overnight (12 h). The solvent, excess anhydride, and trifluoroacetic acid formed were then evaporated under reduced pressure. The trifluoroacetamide obtained was recrystallized from petroleum ether to give TFA-amphetamine-d_0 (mp 58–59 °C), TFA-amphetamine-d_{11} (mp 61 °C), TFA-amphetamine-d_7 (mp 57–58 °C), and TFA-amphetamine-d_6 (mp 58.5–59 °C). GLPC analyses of these TFA derivatives under the same condition as that of free amines gave retention times of 14, 13.5, 13.6, and 13.6 min for TFA derivatives of amphetamine-d_0, -d_{11}, -d_7, and -d_6, respectively.

Pharmacology. The spontaneous locomotor activity of Swiss–Webster albino mice ranging in weight from 22 to 24 g and housed in three circular, six-beam, photocell activity cages (Actophotometer, Metro-Scientific, Inc., Farmingdale, N.Y.) was determined. After the mice were received from the supplier (Scientific Small Animals, Arlington Heights, Ill.) they were acclimated to their new environment for at least 2 weeks. The animals were allowed free access to food and water until placed in activity cages. Two days prior to the experiment, the mice were randomly divided into groups of five mice each, and each mouse was used only once in an experiment. The testing was done between 11:00 a.m. and 5:00 p.m. daily. The amphetamine analogues (27.2 μmol/kg, i.e., 10 mg/kg for amphetamine-d_0, 10.6 mg/kg for amphetamine-d_{11}) as the sulfate salts were dissolved in normal saline and administered intraperitoneally in a volume of 0.2 mL/10 g of body weight. The control groups received a similar volume of normal saline and their spontaneous locomotor activity was determined daily.

Groups of five mice were treated and placed randomly in the activity cages. The number of interruptions of the light beam by the mice was recorded every 10 min in the first hour and then every hour for a period of 5 h. At least three groups of mice were used to determine the spontaneous locomotor activity of each amphetamine analogue. The results of these tests are shown in Table I.

Four analogues of amphetamine, namely, protioamphetamine (S), amphetamine-d_{11} (1), amphetamine-d_7 (2), and amphetamine-d_6 (3), were subjected to toxicity studies, using Swiss–Webster male mice weighing approximately 22 g. The drugs were injected intraperitoneally and the animals were watched for 24 h. Three doses (i.e., 313, 353, and 394 μM/kg) were used in determining the LD_{50} for each compound using 14–22 mice for each dose. Dose–response curves were drawn by linear regression analysis. LD_{50} (mg/kg) were obtained using the method of Litchfield and Wilcoxon.[15]

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β-D-Arabinofuran[1′,2′:4,5]oxazolo-1,3,5-triazine-5-*N*-methyl-4,6-dione and Analogues, Unusually Specific Immunosuppressive Agents

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Sequential treatment of the protected β-D-arabinofuran[1′,2′:4,5]-2-aminooxazoline (**2**) with methyl isocyanate and diimidazole carbonyl afforded the 2,2′-anhydro-β-D-arabinofuranosyl nucleoside, **6**. Deprotection and hydrolysis yielded the corresponding arabinoside. Although the anhydronucleoside exhibited in vitro antiviral activity against herpes simplex type 1, it exacerbated the infection in vivo. Further examination uncovered an in vitro inhibition of the induction of a cell-mediated immune response without cytotoxicity.

As part of a program in our laboratory of examining the chemistry and pharmacology of the antiviral, antibiotic nucleoside, dihydro-5-azathymidine (**1**),[1] we have explored the syntheses of related structural types to expand its spectrum of biological activity. As an example of this, we report that the related 6-oxo arabinoside, **8a**, and the

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