68497-65-4; **16**, 70717-47-4; **17**, 68644-48-4; **18**, 70717-53-2; **19**, 70717-51-0; **19**·HCl, 70717-52-1; *cis*-**20**, 88981-75-3; *trans*-**20**, 88981-76-4; **21**, 68497-80-3; *cis*-**22**, 68497-67-6; **23**, 88981-77-5; **24**, 88981-78-6; *cis*-**25**, 68497-71-2; **26**, 88981-79-7; **27**, 88981-80-0; **28**, 88981-81-1; **29**, 88981-82-2; **30**, 88981-83-3; **31**, 88981-84-4; **32**, 88981-85-5; **33**, 68497-64-3; **34**, 68497-79-0; **35**, 70717-46-3; *cis*-**36**, 88981-86-6; **37**, 88981-87-7; **38**, 7491-74-9; **39**, 77472-70-9; **40**, 88981-88-8; *trans*-41, 88981-89-9; *trans*-**42**, 88981-90-2; **43**, 61516-73-2; *cis*-44, 88981-91-3; *trans*-**44**, 88982-01-8; *cis*-**45**, 88981-92-4; *trans*-**45**, 88982-02-9; *cis*-**45**·2HCl, 88981-93-5; *trans*-**45**·2HCl, 88982-03-0; **46**, 88981-94-6; **47**, 88981-95-7; **48**, 62842-31-3; **49**, 14157-00-7; **50**, 88981-96-8; $H_2N(CH_2)_2N[CH(CH_3)_2]_2$, 121-05-1; $H_2N(CH_2)_2N(CH_3)_2$, 108-00-9; $H_2N(CH_2)_2N[CH_2CH(CH_3)_2]_2$, 14156-98-0; $H_2N(CH_2)_2N(C_2H_5)_2$, 100-36-7; $H_2NC(CH_2)_3N[CH(CH_3)_2]_2$, 53485-05-5; $H_2N(CH_2)_3N(CH_3)_2$, 109-55-7; $H_2N(CH_2)_4N[CH(CH_3)_2]_2$, 13901-39-8; $BrCH_2COOC_2H_5$, 105-36-2; 2-(*cis*-2,6-dimethylpiperidinyl)ethylamine, 1788-35-8; 2-(piperidinyl)ethylamine, 27578-60-5; 3-(2,2,4,6-tetramethylpiperidinyl)propylamine, 13901-37-6; 3-(2-methylpiperidinyl)propylamine, 25560-00-3; 3-(4-morpholinyl)propylamine, 123-00-2; 4-(*cis*-2,6-dimethylpiperidinyl)butylamine, 88981-97-9; 2-(1-piperidinyl)propylamine, 54151-70-1; 2-methyl-2-(1-piperidinyl)propylamine, 54151-73-4; ethyl 2-(2-oxo-1-pyrrolidinyl)propanoate, 70717-55-4; ethyl 2-(2-methyl-5-oxo-1-pyrrolidinyl)acetate, 33927-64-9; ethyl 2-(2-ethyl-5-oxo-1-pyrrolidinyl)propanoate, 88981-99-1; ethyl 2-(2-oxo-4-phenyl-1-pyrrolidinyl)acetate, 70291-40-6; ethyl 2-(2-oxo-1-piperidinyl)-acetate, 22875-63-4; ethyl 3-(2-oxo-1-pyrrolidinyl)propanoate, 61930-87-8; *trans*-2,6-dimethylpiperidine, 10066-29-2; 2-pyrrolidinone, 616-45-5; *cis*-2,6-diethylpiperidine, 88981-98-0; *trans*-2,6-diethylpiperidine, 88982-00-7; acrylonitrile, 107-13-1; 3-phenyl-2-pyrrolidinone, 6836-97-1.

Synthesis of Some Phosphonates with Antiherpetic Activity

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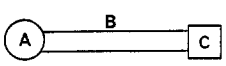
Sterling-Winthrop Research Institute, Rensselaer, New York 12144. *Received August 15, 1983*

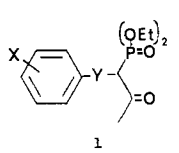
Several keto phosphonates, phosphonoacetates, and dialkyl phosphonates containing (aryloxy)aryl groups were synthesized and evaluated for antiherpteic activity. Two of the most active compounds, **12** and **16**, were evaluated topically in the mouse vaginal model against herpes simplex virus (HSV) type 2. Compound **16** exhibited an increased survival rate, as well as increased survival time. Evaluation of **16** in the guinea pig skin test against HSV-2 produced a reduction in virus titer, as well as in mean vesicle score.

During the past several years, we have been engaged in the synthesis of a class of compounds with antiviral activity, whose structure may be graphically represented by **1**. We have shown the necessity of having an aromatic ring in position A, and thus far, a β-diketone[1-4] or pyrazole ring[5] in position C, the aforementioned being separated by a bridge of five to eight carbon atoms or the equivalent.

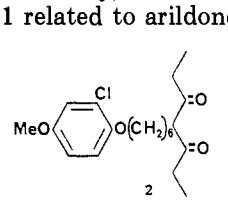


In pursuing this approach, we have synthesized some compounds where C contains a phosphonate group. The presence of the phosphonate group in an antiherpetic agent is not novel, since phosphonoacetic[6] and phosphonoformic acid[7,8] have been investigated as topical agents. Phosphonoacetic acid causes irritation when applied topically[8,9] and has been found to accumulate in bone;[10] however, phosphonoformic acid has been reported to be less irritating.[11]

The introduction of the phosphonate group into our work was based on the isosteric similarity between the phosphonate and ketone groups. Initially, our intent was to prepare keto phosphonates **1** related to arildone (**2**),



1

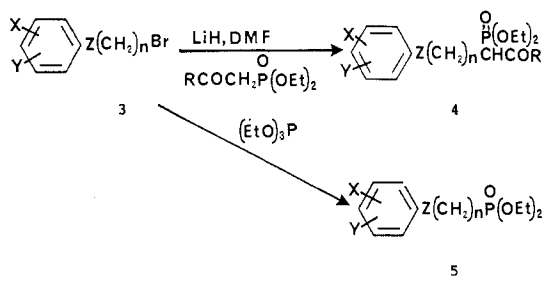


2

which is currently undergoing clinical trials against herpetic infections. However, more simplified structures have been prepared and tested.

Chemistry. Most of the compounds were prepared according to the sequence outlined in Scheme I.

Scheme I



3 4 5

The appropriate bromide **3** was reacted with the lithium salt of a β-keto phosphonate or phosphonoacetic acid ester

†Deceased.

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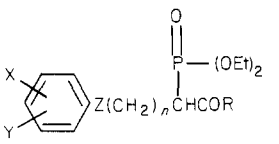
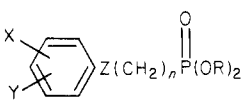
Table I



compd	X	Y	Z	n	R	bp, °C (mm)	yield,[b] %	formula[c]	HSV-2[d] MIC,[a] μg/mL
6	2-Cl	4-CH_3O	O	4	CH_3	*e*	50	$C_{18}H_{28}ClO_6P$	12
7	2-Cl	4-CH_3O	O	5	CH_3	*f*	34	$C_{19}H_{30}ClO_6P$	6
8	2-Cl	4-CH_3O	O	6	CH_3	*f*	36.5	$C_{20}H_{32}ClO_6P$	6
9	2-Cl	4-CH_3O	O	7	CH_3	*f*	31	$C_{21}H_{34}ClO_6P$	6
10	2-Cl	4-CH_3O	O	8	CH_3	*g*	22	$C_{22}H_{36}ClO_6P$	6
11	H	4-CH_3O	CH_2	3	CH_3	*h*	38	$C_{18}H_{29}O_5P$	25
12	2-Cl	4-CH_3O	O	6	C_2H_5O	188-192 (0.05)	72.5	$C_{21}H_{34}ClO_7P$	3

[a] Minimum inhibitory concentration. [b] Yields refer to analytically pure product. [c] Analyzed for C, H, N, or Cl where applicable. [d] Herpes virus. [e] Pure sample obtained by column chromatography on silica and eluted with C_2H_5OH-Et_2O (3:7). [f] Et_2O-THF (60:40). [g] C_2H_5OH-Et_2O (50:50). [h] CH_3OH-Et_2O (10:90).

Table II



compd	X	Y	Z	n	R	mp or bp (mm), °C	yield, %	formula[c]	HSV-2[d] MIC, μg/mL
13	2-Cl	4-CH_3O	O	3	C_2H_5	160-162 (0.01)	65	$C_{14}H_{22}ClO_5P$	inact
14	2-Cl	4-CH_3O	O	4	C_2H_5	160-162 (0.01)	73	$C_{15}H_{24}ClO_5P$	inact
15	2-Cl	4-CH_3O	O	5	C_2H_5	193-195 (0.08)	32.5	$C_{16}H_{26}ClO_5P$	inact
16	2-Cl	4-CH_3O	O	6	C_2H_5	195-197 (0.005)	39	$C_{17}H_{28}ClO_5P$	3
17	2-Cl	4-CH_3O	O	7	C_2H_5	194-195 (0.03)	39	$C_{18}H_{30}ClO_5P$	inact
18	2-Cl	4-CH_3O	O	8	C_2H_5	207-209 (0.8)	33	$C_{19}H_{32}ClO_5P$	inact
19	2-Cl	4-CH_3O	O	6	C_3H_7	220-224 (0.005)	68	$C_{21}H_{36}ClO_5P$	6
20	2-Cl	4-CH_3O	O	6	H	102-104[a]	33	$C_{13}H_{20}ClO_5P$	inact
21	H	4-$COOC_2H_5$	O	6	C_2H_5	190-196 (0.2)	51	$C_{19}H_{31}O_6P$	6
22	H	4-COONa	O	6	C_2H_5	215-216[b]	76	$C_{17}H_{26}NaO_6P$	inact
23	H	4-Br	O	6	C_2H_5	165-180 (0.05)	45	$C_{16}H_{26}BrO_4P$	inact
24	2-CH_3O	6-CH_3O	O	6	C_2H_5	180-190 (0.05)	54.5	$C_{18}H_{31}O_6P$	inact
25	H	4-Cl	O	6	C_2H_5	180 (0.1)	63	$C_{16}H_{26}ClO_4P$	inact
26	2-Cl	6-Cl	O	6	C_2H_5	160-170 (0.07)	32	$C_{16}H_{25}Cl_2O_4P$	12
27	H	4-AcO	O	2	C_2H_5	137-147 (0.05)	48	$C_{14}H_{21}O_6P$	inact
28	H	4-OH	O	2	C_2H_5	94-95[b]	73	$C_{12}H_{19}O_5P$	inact

[a] Recrystallized from ethyl acetate-pentane. [b] Recrystallized from ether. [c] Analyzed for C, H, N, or Cl where applicable. [d] Herpes virus.

in DMF to give **4**. The diethyl phosphonates **5** were prepared by heating the bromides **3** with triethyl phosphite at 180–190 °C.

Biological Results

The in vitro evaluation of the compounds in Tables I and II was performed according to the procedures previously described.[1] Rather than vary the substituents on the phenyl ring, we selected the 2-chloro-4-methoxyphenyl group, since we had established the importance of this moiety in previous series. Varying the length of the alkyl group (Table I) did not appreciably affect the level of activity, although the butyl homologue **6** exhibited slightly less activity than the higher homologues (**7–10**). The highest level of activity was shown where the ketone was replaced with an ester group (**12**).

Table III. Comparative Effect of Compounds 12 and 16 on the Survival Rate of Mice Iinfected Intravaginally with Herpes Simplex Virus Type 2

compd	daily dose (b.i.d.),[a,b] %	% survival	av. survival time, days
12	10	20	>8.7
	5	10	>7.3
	2.5	10	>8.1
16	10	60	>11.3
	5	50	>10.5
	2.5	30	>10.1
placebo		30	8.4

[a] Administered intravaginally in gum tragacanth. [b] Treatment initiated 4-h postinfection and continued for 7 days.

We next removed the carbonyl moiety entirely (Table II). Interestingly, this series was not as uniformly active as the keto phosphonate series, and variations in the length of the chain did influence the level of activity. When the bridge was varied from three to eight carbon atoms (compounds **13–18**), only the C-6 homologue **16** exhibited ac-

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Table IV. Effect of 5 and 10% of Compound 16 in a Vanishing Cream Base on the Virus Titer in a Herpetic Skin Infection of Guinea Pigs Employing Herpesvirus Hominis, Type 1, AA Strain[a]

	virus titer, log $TCID_{50}$/mg of protein				
	days postinfection				
concn	day 1	day 2	day 3	day 4	day 5
10% 16	3.18	<1.34	<1.60	<1.60	<1.25
5% 16	3.94	<1.41	<1.14	<1.25	<1.11
placebo	3.73	3.86	3.79	3.03	3.01
nonmedicated	3.99	4.06	3.79	3.50	3.73

[a] Least significant difference between two treatment means within a time period is 0.60 at $p = 0.05$, and 0.80 at $p = 0.01$.

Table V. Statistical Analysis of the Effect of 5 and 10% of Compound 16 in a Vanishing Cream Base Applied Five Times Daily for 4 Days on the Development of Herpetic Vesicles in Guinea Pigs Infected Intradermally with Herpesvirus Hominis, Type 1, AA Strain[a]

	mean vesicle score				
	days postinfection				
concn	day 1	day 2	day 3	day 4	day 5
10% 16	0.67	0.83	0.91	0.92	1.1
5% 16	0.75	1.17	0.75	1.0	0.67
placebo	0.67	1.6	1.8	2.2	2.6
nonmedicated	0.83	1.4	1.8	2.4	2.8

[a] Treatment started 24 h postinfection. Least significant difference between two treatment means within a time period at $p = 0.05$ is 0.42, and at $p = 0.01$ is 0.56.

tivity. The corresponding propyl ester **19** was equally active.

Some variations were made in the substituents on the phenyl ring. The only compound with noteworthy activity was the 4-carbethoxy homologue **21**.

Compounds **12** and **16**, in gum tragacanth, were evaluated intravaginally against Herpes simplex virus type 2 in the mouse genital model according to the procedure previously described.[5] The results are shown in Table III. Compound **16** demonstrated a dose–response with a 60% survival rate at the 10% level of drug and an average survival of 11.3 days as compared to 8.4 days for the placebo-treated animals. Compound **12** exhibited no effect at any of the levels tested.

In view of the results of the mouse test, compound **16** was evaluated topically against herpes virus type I in the guinea pig skin model, according to the procedure described in the Experimental Section. The compound was administered as a 5 and 10% cream. The effect on the virus titer is shown in Table IV. Both 5 and 10% concentrations of the drug dramatically reduced the amount of measurable virus in the lesions to essentially 0 when measured 24 h postmedication or 48 h postinfection. This effect was sustained throughout the test. The effect on mean vesicle score is shown in Table V. Whereas the mean vesicle scores of the placebo-treated and nonmedicated animals increased over the 5 days of sampling, those of the 5 and 10% concentrations remained essentially constant (within experimental error).

A comparison was made between compound **16** in a 5% cream base, a 5% acyclovir cream, and an 8% arildone cream. The reduction in virus titer is shown in Table VI. Compound **16** effected a far greater reduction in virus titer than both acyclovir and arildone. A corresponding reduction in the mean vesicle score was observed for all three drugs, with compound **16** demonstrating the greatest effect (Table VII).

Discussion

In the introduction of this paper, we have presented a simple model that we envisioned as fulfilling certain requirements for antiviral activity. The phosphonates that we have prepared fit this model as demonstrated by the results of the structure–activity study that was discussed. From this paper and our previous publications, it is apparent that the distance requirement between A and C of our model is approximately the same regardless of the nature of C, within the limitations of our studies.

In view of these results, one might speculate that those compounds that fit our proposed model might somehow fulfill (1) structural requirements, (2) spatial requirements, (3) electronic requirements, and/or (4) lipophilic requirements.

Experimental Section

Melting points were run according to the USP procedure and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.4% of the theoretical values. Analyses were performed by Instranal Laboratories, Rensselaer, NY, and Galbraith Laboratories, Knoxville, TN. NMR spectra were determined on a Varian HA-100 spectrometer, and the mass spectra were determined on a Jeolco double-focusing high-resolution mass spectrometer by S. Clemans. Acyclovir was obtained as 5% Zorivax cream ointment from Burroughs Wellcome.

Diethyl [1-Acetyl-4-(2-chloro-4-methoxyphenoxy)butyl]-phosphonate (6). A mixture of 790 mg (0.1 mol) of LiH and 19.4 g (0.1 mol) of diethyl 2-oxopropylphosphonate in 350 mL of DMF was heated to 80 °C for 3 h. After the mixture was cooled, 34 g (0.1 mol) of 1-(2-chloro-4-methoxyphenoxy)-4-iodobutane[2] in 25 mL of DMF was added, and the solution was heated to 55–60 °C for 2 days. The solvent was removed in vacuo, and the residual oil was partitioned between benzene and water. The organic layer was separated, washed, and dried. Removal of the solvent gave 30.8 g of oil, which was subjected to column chromatography on silica gel and eluted with 30% ethanol–70% ether, v/v, and 11.5 g of oil was obtained. Anal. ($C_{18}H_{28}ClO_6P$) C, H, Cl.

Ethyl 8-(2-Chloro-4-methoxyphenoxy)-2-(diethoxyphosphinyl)octanoate (12). To a solution of 3.5 g (0.156 mol) of triethyl phosphonoacetate in 10 mL of xylene was added, in small portions, 623 mg (0.0159 mol) of potassium After the addition was complete, the mixture was heated to reflux for 1 h, and then 5 g (0.0156 mol) of 6-(2-chloro-4-methoxyphenoxy)hexyl bromide[2] in 5 mL of xylene was added. The resulting solution was refluxed for 5 h and then filtered, after cooling, and the filtrate

Table VI. Effect of 5% Compound 16, 5% Acycloguanosine, and 8% Arildone on the Growth of Herpes Simplex Type 1 on the Skin of Guinea Pigs Infected Intradermally[a]

	log geometric mean pfu[b]/mg of protein (±SE) on the following days			
medication	1	2	3	4
5% 16	4.23 (±0.24)	0.96[c]	0.79[c]	0.76[c]
5% acycloguanosine[d]	4.30 (±0.53)	4.09 (±0.30)	3.31 (±0.52)	2.13 (±0.60)
8% arildone	4.13 (±0.33)	4.07 (±0.19)	2.92 (±0.14)	2.62 (±0.51)
placebo	3.83 (±0.36)	3.63 (±0.60)	2.67 (±0.31)	2.62 (±0.20)
nonmedicated	3.94 (±0.50)	4.16 (±0.36)	3.25 (±0.29)	3.06 (±0.27)

[a] Medication applied topically 5 times a day, starting 24 h postinfection. [b] pfu = plaque forming units. [c] An arbitrary value. No detectable plaques. [d] Commercial ointment.

Table VII. Statistical Analysis of the Effect of 5% Compound 16, 5% Acycloguanosine, and 8% Arildone Applied Five Times Daily for 4 Days on the Development of Herpetic Vesicles in Guinea Pigs Infected Intradermally with Herpesvirus Hominis, Type 1, AA Strain[a]

medication	mean vesicle score on the following days postinfection			
	day 1	day 2	day 3	day 4
5% 16	0.5	0.75	0.50	0.42
5% acycloguanosine[b]	0.58	0.83	1.16	1.0
8% arildone	0.33	0.83	0.92	0.83
placebo	0.66	1.50	1.75	1.42
nonmedicated	0.66	1.33	1.58	1.66

[a] Treatment started 24 h postinfection. [b] Commercial ointment.

was concentrated to dryness. The residual oil was distilled: yield 3.7 g (72.5%); bp 188–192 °C (0.04 mm). Anal. ($C_{21}H_{34}ClO_7P$) C, H, Cl.

Diethyl [2-(4-Acetoxyphenoxy)ethyl]phosphonate (27). A solution of 10.3 g (0.4 mol) of 2-(4-acetoxyphenoxy)-2-bromoethane and 6.6 g (0.04 mol) of triethyl phosphite was heated to 180–190 °C for 2 h and then heated to 55–60% °C for 2 days. The solvent was removed in vacuo, and the residual oil was partitioned between benzene and water. The organic layer was separated, washed, and dried. Removal of the solvent gave 30.8 g of oil, which was subjected to column chromatography on silica gel and eluted with 30% ethanol–70% ether, v/v, and 11.5 g of oil was obtained. Anal. ($C_{18}H_{28}ClO_6P$) C, H, Cl.

Diethyl [2-(4-Hydroxyphenoxy)ethyl]phosphonate (28). To a solution of 8 g (0.025 mol) of **27** in 8 mL of CH_2OH was added 80 mL of 40% $(CH_3)_2NH$. The solution was stirred for 2 h at room temperature and then evaporated in vacuo. The residual oil was partioned between 300 mL of $(C_2H_5)_2O$ and 50 mL of H_2O. The organic layer was washed and dried, and the solvent was removed, leaving a solid. The material was recrystallized from $(C_2H_5)_2O$: yield 5 g; mp 94–95 °C. Anal. ($C_{12}H_{19}O_5P$) C, H, P.

Diethyl [6-(2-Chloro-4-methoxyphenoxy)hexyl]-phosphonate (16). A solution of 10 g (0.03 mol) of 6-(2-chloro-4-methoxyphenoxy)hexyl bromide in 5.2 g (0.03 mol) of triethyl phosphite was heated to 180–190 °C, and the ethyl bromide formed was allowed to distill. After 2 h, the solution was distilled in vacuo: yield 4.6 g (39%); bp 195–197 °C (0.005 mm). Anal. ($C_{17}H_{28}ClO_5P$) C, H, Cl.

Guinea Pig Skin Infection with Herpes Virus Type 1. Albino guinea pigs, Hartly strain, weighing 350–400 g were infected with undiluted Herpesvirus hominis type 1, AA strain. An area 12 mm in diameter was marked on one epilated flank and 0.05 mL of an undiluted virus suspension was placed in the circle and injected intradermally with a Sterneedle vaccination gun. Starting 24 h postinfection, medication was applied five times daily for 4 days by gently massaging approximately 0.2 mL of the appropriate drug in a cream formulation or placebo cream into the site of infection with a fresh finger cot over rubber gloves.

Evaluation of Clinical Results. The animals were scored by a person not involved in the medication process in order to eliminate bias in evaluating the clinical effects. Guinea pigs were scored on the basis of severity of herpetic vesicles, by using 0–3 range in 0.5 increment. The scores were recorded in a notebook but not examined by the scorer until the experiment was terminated. The data was analyzed by an analysis of variance.

Lesion Sampling for Virus Content. Six guinea pigs from each group were euthanized daily for 5 consecutive days starting 24 h postinfection. The site was scraped vigorously with a sterile disposable scalpel, and recovered material was placed in a Kontes glass tissue grinder tube containing 4.0 mL of a balanced salt solution in an ice bath. The scrapings were triturated and sedimented by low-speed centrifugation. The opalescent supernatant was divided into three aliquots and stored at −70 °C for virus titer determination and protein analysis.

Virus Quantitation Assay. Monolayers of BSC-1 cells were prepared in Costar cluster dishes, each dish containing six wells of 35-mm diameter. The supernatants prepared from the skin scrapings were thawed and diluted in Eagles medium supplemented with 2% fetal calf serum from 10^{-1} to 10^{-3}. One milliliter of each dilution was added to the wells (in triplicate) after the growth medium was removed. The virus was allowed to adsorb for 1 h at 37 °C in a 5% CO_2 atmosphere, after which the residual material was removed, and 5.0 mL of a mixture of equal parts of 2X medium 199 supplemented with 5% fetal calf serum and 1% agarose (Oxoid, agar no. 1), maintained at 43 °C, was added. The agar was allowed to gel, and the dishes were then incubated at 37 °C in a 5% CO_2 atmosphere for 4 days. At the end of the incubation period, the cells were fixed to the surface of the well with 1.0 mL/well of 1% formalin containing 0.2% sodium acetate and stored at 4 °C for 24 h. The agar was gently removed from the wells, the monolayers were stained with a solution of Crystal violet in formalin, and the plaques were counted. The minimal amount of drug carried over the virus quantitation assay was shown to have no effect on plaque formation.

Registry No. **3** (X = H; Y = 4-AcO; Z = O; n = 2), 89210-89-9; **3** (X = 2-Cl; Y = 4-CH_3O; Z = O; n = 6), 56219-58-0; **6**, 73515-00-1; **7**, 73514-99-5; **8**, 73515-01-2; **9**, 73514-97-3; **10**, 73514-98-4; **11**, 73514-96-2; **12**, 73514-95-1; **13**, 89210-90-2; **14**, 89210-91-3; **15**, 89210-92-4; **16**, 73514-87-1; **17**, 73514-91-7; **18**, 73514-90-6; **19**, 73514-88-2; **20**, 89210-93-5; **21**, 73515-02-3; **22**, 73514-93-9; **23**, 89210-94-6; **24**, 89210-95-7; **25**, 89210-96-8; **26**, 73514-92-8; **27**, 89210-97-9; **28**, 89210-98-0; 1-(2-chloro-4-methoxyphenoxy)-4-iodobutane, 73523-71-4; diethyl 2-oxopropyl phosphonate, 1067-71-6; triethyl phosphonoacetate, 867-13-0; triethyl phosphite, 122-52-1.

Substituent Effects on Reactivity and Spectral Parameters of Cephalosporins[1]

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The chemical reactivity of a series of cephalosporins is examined as a function of the substituents at positions 3 and 7. In most cases, the nature of the C_7 side chain has a minor influence on the β-lactam reactivity. But in the case of amino-containing C_7 substituents, when intramolecular nucleophilic attack may occur, the reactivity may be greatly increased. The spectroscopic and structural characteristics of the β-lactam linkage do not correlate with the chemical reactivity of studied compounds. The hydrolysis rates are linked neither with the IR frequency or ^{13}C NMR chemical shift of the carbonyl β-lactam nor with the geometry of the β-lactam ring. However, a relationship is confirmed between the β-lactam ring opening rate and the polarity of the C_3–C_4 double bond, reflected in the different ^{13}C NMR chemical shifts of those atoms. The results are an experimental verification of the theoretical calculations of Boyd et al. on cephalosporin model compounds, which foresee that a C_3 substituent could favor the opening of the β-lactam cycle by stabilizing a transition state involved in alkaline hydrolysis.

The penicillins and cephalosporins are β-lactam antibiotics that inhibit the peptidoglycan transpeptidation step by inactivating certain enzymes involved in the synthesis of bacterial cell walls. Because of the structural analogy

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