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Serotonin Receptor Binding Affinities of Tryptamine Analogues

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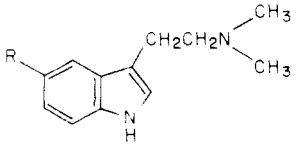
Using a rat fundus model, the serotonin (5-HT) receptor binding affinities of 27 tryptamine analogues were determined. Factors which might affect affinity were examined, e.g., lipid solubility, as reflected by partition coefficient, and pK_a. Structure–activity relationships were developed and are discussed in terms of substituents on the terminal amine, the side chain, and the indole 1 position, the 5 position, and at other positions on the indolic nucleus. If lipid solubility and metabolism can be accounted for, there appears to be a parallelism between 5-HT receptor binding affinities and the hallucinogenic (psychotomimetic) potencies of several of these compounds.

As first suggested by Woolley, there may be a relationship between the hallucinogenic (psychotomimetic) activity displayed by various *N,N*-dialkyltryptamine derivatives and their ability to interact with serotonin (5-hydroxytryptamine, 5-HT) receptors in the brain.[1,2] The possible endogenous production of such compounds, via abnormal 5-HT metabolism, might also be related to some of the symptoms of mental illnesses;[2] i.e., various aspects of mental illness and the mechanism of action of the hallucinogenic *N,N*-dialkyltryptamines may share certain common components. In support of this theory, enzyme systems have now been identified which can convert tryptamine and 5-HT into *N,N*-dimethyltryptamine (DMT, **1**), 5-hydroxydimethyltryptamine (bufotenine, **2**)



1, R = H
2, R = OH
3, R = OCH_3

and 5-methoxydimethyltryptamine (**3**).[3,5] In addition, attempts have been made to detect these compounds in the urine of schizophrenic patients. While there have been reports that schizophrenics may possess significant levels of the above-mentioned alkylated tryptamines,[6,7] these findings are still controversial.[8,9] For a review see ref 10. Nevertheless, it has been demonstrated that these hallucinogens can interact with serotonergic receptors. Aghajanian and Haigler, employing a microiontophoretic technique, have concluded that low doses of hallucinogenic tryptamines act preferentially upon presynaptic 5-HT receptors to inhibit raphe neurons.[11] Bennett and Snyder, on the other hand, have investigated the binding of tryptamines to calf brain membrane preparations and have suggested that the binding sites involved might be postsynaptic 5-HT receptors.[12,13]

The potencies of tryptamines in causing contractions of the isolated rat stomach fundus strip paralleled their potencies in blocking lysergic acid diethylamide (LSD) binding by rat brain homogenates; thus, the fundus strip appears to be a valid model for brain receptors.[14] An investigation of the structure–activity relationships of tryptamine derivatives which interact with 5-HT receptors of various tissue preparations, for example, the fundus strip, might shed light on the structural requirements of these receptors. Furthermore, such an investigation might assist in elucidating the mechanism of action of hallucinogenic tryptamines in as much as the 5-HT receptors of these preparations might serve as models for central 5-HT–receptor interactions. We have previously reported that analogues **1–3** possess high binding affinities for the 5-HT receptors of the rat fundus preparation.[15] In this present study, we have determined the binding affinities (pA_2 values) of a rather extensive series of *N,N*-dialkyltryptamines and related compounds for the 5-HT receptors of this same model system in order to delineate SAR and

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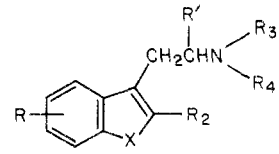
Table I. Binding Affinity Data[a]



no.	compd	R	X	R_2	R_3	R_4	R'	pA_2[b]	n[c]
2	bufotenine	5-OH	NH	H	Me	Me	H	7.41 (±0.05)	2
3	5-MeO-DMT	5-OMe	NH	H	Me	Me	H	7.08 (±0.10)	5
4	5-MeO-DET	5-OMe	NH	H	Et	Et	H	6.94 (±0.06)	2
5	5-MeT	5-Me	NH	H	H	H	H	6.86 (±0.08)	3
6	5-MeO-MET	5-OMe	NH	H	Me	Et	H	6.85 (±0.05)	2
7	psilocin	4-OH	NH	H	Me	Me	H	6.84 (±0.16)	4
8	5-MeO-DPT	5-OMe	NH	H	Pr	Pr	H	6.53 (±0.05)	2
9	5-Me-DMT (5-TMT)	5-Me	NH	H	Me	Me	H	6.52 (±0.15)	2
10	7-Me-DMT (7-TMT)	7-Me	NH	H	Me	Me	H	6.29 (±0.08)	2
11	4-NH_2-*N*,*N*-DMT	4-NH_2	NH	H	Me	Me	H	6.28 (±0.07)	2
12	tryptamine	H	NH	H	H	H	H	6.27 (±0.17)	3
13	α-MeT	H	NH	H	H	H	Me	6.25 (±0.15)	5
14	4-MeO-DMT	4-OMe	NH	H	Me	Me	H	6.17 (±0.01)	2
15	2-Me-DMT	H	NH	Me	Me	Me	H	6.04 (±0.08)	2
16	*S*-DMT[e]	H	S	H	Me	Me	H	6.03 (±0.03)	2
17	1-Me-DMT (1-TMT)	H	NMe	H	Me	Me	H	6.02 (±0.08)	3
18	DMHT[d]	H	NH	H	Me	Me	H	6.00 (±0.07)	2
1	DMT	H	NH	H	Me	Me	H	6.00 (±0.08)	2
19	5-Ac-DMT	5-Ac	NH	H	Me	Me	H	5.86 (±0.06)	2
20	DET	H	NH	H	Et	Et	H	5.79 (±0.03)	2
21	6-MeO-DMT	6-OMe	NH	H	Me	Me	H	5.77 (±0.01)	2
22	C-DMT[f]	H	CH_2	H	Me	Me	H	5.68 (±0.06)	2
23	N^1-Me-N^4-[2-(3-indoyl)Et]Pip[g]	H	NH	H -$CH_2CH_2N(CH_3)CH_2CH_2$-			H	5.55 (±0.06)	2
24	5,7-MeO_2-DMT	5,7-OMe_2	NH	H	Me	Me	H	5.50 (±0.04)	2
25	7-MeO-DMT	7-OMe	NH	H	Me	Me	H	5.33 (±0.02)	2
26	α-EtT	H	NH	H	H	H	Et	5.23 (±0.14)	3
27	7-OHDMT	7-OH	NH	H	Me	Me	H	4.88 (±0.08)	2

[a] 5-HT: $pD_2 = 7.45$ (±0.25); $n = 104$. [b] Values are plus or minus standard deviation. [c] Number of determinations. [d] Compound 18 is dimethylhomotryptamine [3-(dimethylamino-*n*-propyl)indole]. [e] 16 is 3-(2-dimethylaminoethyl)benzothiophene. [f] 22 is 3-(2-dimethylaminoethyl)indene. [g] 23 is N^1-methyl-N^4-[2-(3-indoyl)ethyl]piperazine.

to determine if a relationship exists between pA_2 and the hallucinogenic potency of these compounds.

Results

The binding affinity data are reported in Table I. Antagonism appears to be competitive, as noted by parallel dose–response curves in the absence and in the presence of increasing concentrations of compound. Of the compounds examined, bufotenine (**2**) has the highest apparent affinity for the 5-HT receptors of the rat stomach fundus. In general, it appears that a hydroxy or methoxy group at the 5 position greatly enhances affinity. Moving the methoxy group of 5-methoxydimethyltryptamine (**3**), for example, to the 4, 6, or 7 position, results in a decrease in affinity, as does replacement of the indolic nitrogen by an sp^3 hybridized carbon atom. Comparing DMT with compounds **18** and **16**, it appears that extending the side chain by one methylene unit or replacing the indole nitrogen by a sulfur atom has no effect on affinity.

In order to determine whether or not pK_a or lipid solubility, as reflected by partition coefficient, has any effect on affinity, a small series of compounds was examined. It can be seen from the data in Table II that there is no direct relationship between affinity and either the pK_a or the chloroform–aqueous buffer partition coefficients of these compounds.

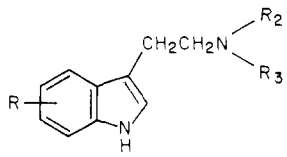
Discussion

Of the various isolated tissue preparations on which responses to 5-HT and related agonists have been studied, the isolated rat fundus preparation of Vane[16] is perhaps the most sensitive and the most extensively employed.[17] In an earlier publication, it was reported that more than one type of contractile tryptamine receptor is present on the rat fundus, i.e., 5-HT receptors and PRT (or phenoxybenzamine-resistant tryptamine) receptors.[18] In light of the discovery of the PRT receptors, previous structure–activity results derived from rat fundus data, particularly those derived from investigations of agonism, may now be subject to a different interpretation. For example, Vane[19] found DET (**20**) to be a more potent agonist than DMT (**1**); as shown in Table I, the affinity of DMT for the 5-HT receptors is approximately twice that of DET. The results obtained by Vane[19] may be explained on the basis of different intrinsic activities or may be reflective of a differential drug–PRT receptor interaction. Determination of affinities (pA_2 values), by virtue of the manner in which they are obtained, obviates PRT receptor involvement. In other words, rather than studying the agonistic effect of these compounds, their ability to block the interaction of 5-HT with its own receptors is being measured.

As might be expected, those compounds which possess the greatest structural similarity to 5-HT also possess the highest affinity for the 5-HT receptors of the model system. For purposes of discussion, five major structural features can be considered and each will be addressed separately: (a) the terminal amine function, (b) the side chain, (c) the indole 1 position, (d) the 5-position substituents, and (e) other substituents.

It has been suggested that the terminal amine group of 5-HT interacts with the receptor in an electrostatic manner.[17] The compounds in Table II possess similar pK_a

Table II. Partition Coefficients and pK_a Values of Several *N,N*-Dialkyltryptamines



no.	R_1	R_2	R_3	partit coeff[a,b]	pK_a[c] (37 °C)
2	5-OH	CH_3	CH_3	0.06	
3	5-OCH_3	CH_3	CH_3	3.30	9.41 (±0.07), 7
4	5-OCH_3	C_2H_5	C_2H_5	7.89	9.84 (±0.02), 5
6	5-OCH_3	CH_3	C_2H_5	5.72	9.73 (±0.06), 6
7	4-OH	CH_3	CH_3	3.30	
14	4-OCH_3	CH_3	CH_3	2.28	9.41 (±0.05), 7
1	H	CH_3	CH_3	2.61	9.33 (±0.06), 7
20	H	C_2H_5	C_2H_5	6.12	9.61 (±0.03), 6
21	6-OCH_3	CH_3	CH_3	3.68	9.33 (±0.03), 6
25	7-OCH_3	CH_3	CH_3	9.54	9.10 (±0.04), 14

[a] Ref 25. [b] Chloroform-aqueous buffer (pH 7.4) partition coefficients; P. K. Gessner, unpublished data. [c] pK_a values are plus or minus standard deviation and are followed by number of point determinations.

values but differ primarily in respect to the steric bulk about this terminal amine. In comparing the affinity of DMT (**1**) to that of DET (**20**) or in comparing the affinities of the 5-methoxy analogues of DMT, DET, and DPT (compounds **3**, **4**, and **8**, respectively), as the steric bulk around the terminal amine increases, affinity decreases. Conversion of *N,N*-dimethyltryptamines to tryptamines usually results in a twofold increase in affinity, comparing **5** and **12** with **9** and **1**, respectively. α-Methylation has no apparent effect on binding affinity, comparing the affinity of **12** with that of racemic **13**. The effect of N-dimethylation and α-methylation on affinity parallel that recently reported by Glennon et al.[20] for a series of phenalkylamine analogues. α-Ethylation, on the other hand, results in a decrease in affinity (compare **12** with **26**).

Offermier and Ariens have already determined that the terminal amine is a prerequisite function in order for the tryptamine analogues to possess an affinity for 5-HT receptors.[17] It has been further shown that decreasing the length of the side chain, of DMT for example, from two to one methylene unit decreases affinity.[17] As revealed in Table I, increasing the chain length of DMT (**1**) by one methylene unit to DMHT (**18**) has no effect on affinity. Perhaps by varying the conformation of the propylamine side chain, the terminal amine function of DMHT (**18**) can approximate that distance from the indole ring which is usually achieved by the terminal amine of DMT (**1**).

The conclusion has been reached that an unsubstituted indolic nitrogen is not necessary in order for the tryptamines to bring about various pharmacological responses. Comparing DMT (**1**) with its *N*-methyl analogue **17**, there is virtually no difference in 5-HT receptor affinity as determined in this model system. Winter and Gessner[18] have concluded that the hydrogen of the indolic NH is not a binding site for these compounds on the 5-HT receptors of the rat stomach fundus. In support of this finding, substitution of the NH of DMT by a sulfur atom (compound **16**) has no effect on affinity. Looking at the indene analogue **22**, substitution of the NH by a CH_2 does result in a twofold decrease in affinity. Further work will be necessary to determine if this decrease in affinity is due to an alteration of the electronic nature of the ring or whether it is simply a steric effect.

It has been recently suggested that the phenolic oxygen function at the 5 position of the tryptamine nucleus of 5-HT is not an essential binding site.[21] The affinity of **1**, **5**, and **7**, for example, support this suggestion, although compounds with the highest binding affinities are consistently substituted at the 5 position with either a hydroxy or a methoxy group. Kang and Green have reported a correlation between the agonistic activity of a series of tryptamines, using this same model system, and the resonance constant of the substituent at the 5 position.[22] In comparing the 5-position substituent in compounds **2**, **3**, **9**, and **19**, as electron-donating ability decreases, so does affinity. It appears, then, that rather than (or in addition to) binding with the receptor directly, the 5-position substituent may affect affinity by altering the electronic nature of the indole nucleus.

With respect to substituents at other positions, 4-hydroxy (**7**) and 4-amino (**11**) substitution appear to somewhat enhance the affinity of DMT. Substitution of methoxy groups at the 6 position and in particular at the 7 position has an adverse effect on affinity. We have previously suggested that there may be a hydrophobic site in the 7-position region of the 5-HT receptors of the rat fundus.[20] This would account for the higher affinity of 7-methyldimethyltryptamine (**10**) as compared to DMT itself. It might be argued that incorporation of a methyl group simply enhances the lipid solubility of the molecule in general. However, methylation at the 1 and 2 positions, compounds **17** and **15**, respectively, have no effect on affinity. Furthermore, if a specific hydrophobic site exists, introduction of a polar substituent at the 7 position would be expected to have a detrimental effect on affinity. This is found to be the case, 7-hydroxydimethyltryptamine (**27**) is found to possess the lowest affinity of any compound thus far examined.

Little is known about the hallucinogenic potential of most of the compounds in Table I. However, for those few compounds for which human data are available (i.e., **3**, **7**, **13**, **1**, **20**[29,38]), activity parallels binding affinity rather closely. As activity decreases, so does affinity. This same parallelism is observed when the behavioral effects of **3**, **13**, **14**, **21**, and **25** are compared using trained rats (unpublished observation). Though 5-HT–receptor interactions may be implicated as being involved in the behavioral effects produced by these compounds, it might be incorrect to assume that other factors are not important. For example, based upon the above parallelism, there are several compounds which might be expected to display psychotomimetic properties, such as bufotenine (**2**) and tryptamine (**12**), for which human data are confounding. Although bufotenine, which possesses the highest affinity of those compounds listed in Table I, has been reported to be weakly active in man,[23] this report has been questioned.[24] It has been suggested that bufotenine is not lipid soluble enough to penetrate the blood–brain barrier[25] and the data in Table II would tend to support this. However, Gessner and Dankova have administered 5-acetoxybufotenine to animals where it is hydrolyzed, presumably via brain tissue esterase, to bufotenine. Relating LSD-like effects to total brain levels, they have found the following order of potency: bufotenine > 5-MeODMT > DMT.[26,27] Though not without central and behavioral effects,

tryptamine (12) is not considered to be hallucinogenic in man.[28] Yet, the affinity of tryptamine (12) is twice that of DMT (1). Lacking *N*-alkyl groups, tryptamine perhaps undergoes oxidative deamination more rapidly than DMT.[29] If oxidative deamination is hindered by introduction of an α-methyl group, not only is affinity relatively unaffected (comparing 12 with 13) but 13 is found to possess almost twice the hallucinogenic potency of DMT.

Serotonin receptor binding affinities, alone, are not sufficient to account for the hallucinogenic potential of tryptamine analogues. Other factors need to be taken into consideration, such as lipid solubility (as with bufotenine) and metabolism (as with α-methyltryptamine compared with tryptamine). Nevertheless, these binding-affinity data indicate that further work in this area is warranted.

Experimental Section

Nuclear magnetic resonance (^{1}H NMR) spectra were recorded using a Perkin-Elmer R-24 spectrometer with Me_4Si as an internal standard. Infrared spectra were obtained on a Perkin-Elmer 257 spectrophotometer. Elemental analysis was performed by Atlantic Microlab Inc., Atlanta, GA. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Compounds 4, 6, 8, 10, 14, 21, 22, and 25 were prepared or obtained as we have previously described.[25,30] Compounds 9 and 13[32] were synthesized according to literature procedures. Compound 19 was a gift from M. von Strandtman, WLRI, 7 was from NIDA, and 11, 24, and 27 were from the Psychopharmacology Research Branch of NIMH. Psilocin (7) was used as the free base, 9 and 15 were used as maleate salts, 4, 5, 12, 13, and 24 were used as HCl salts, 26 was used as the acetate, and the remainder were used as hydrogen oxalate salts. The free base of compounds 11, 18, 19, and 22 was initially dissolved in a molar equivalent of dilute HCl solution prior to further dilution.

pK_a values were determined at 37 °C by the titrimetric method of Albert and Serjeant[33] using a Corning Model 10 pH meter. Potassium hydroxide (0.01 N) was used as titrant.

2-Methyl-*N*,*N*-dimethyltryptamine (15). Compound 15, though previously reported, has not been heretofore isolated and characterized. Compound 15 was prepared as described;[34] however, the oily product crystallized upon trituration with hexane. Recrystallization from a benzene–hexane mixture afforded 15 as small white needles, mp 70–71 °C. Dropwise addition of an Et_2O solution of this product to an Et_2O solution of maleic acid resulted in a nearly quantitative yield of the maleate salt. Recrystallization from absolute EtOH gave 15 maleate as a white crystalline product, mp 124–125 °C (lit.[34] mp 127–128 °C).

1-Methyl-*N*,*N*-dimethyltryptamine (17). *N*,*N*-Dimethyltryptamine (0.3 g, 1.6 mmol) was added to a mixture of crushed KOH (0.36 g) in Me_2SO (5 mL) and stirred for 5 min. Following the addition of MeI (0.23 g, 1.6 mmol), stirring continued for 1 h; 2.5 mL of H_2O was added and the mixture was stirred an additional 10 min. After extraction of the mixture with Et_2O (3 × 5 mL), the combined extracts were dried ($CaCl_2$) and evaporated in vacuo to give approximately 100 mg of a crude yellow oil. A small portion of the product was converted to the picrate, mp 175–178 °C (lit.[35] 175–177 °C), while the remainder was dissolved in Et_2O and added dropwise to an Et_2O solution of oxalic acid. The precipitate was collected and recrystallized from 95% EtOH to give 17 hydrogen oxalate, mp 174–176 °C.

***N*^{1}-Methyl-*N*^{4}-(3-indolylglyoxalyl)piperazine (28).** A solution of oxalyl chloride (3.2 g, 25.2 mmol) in anhydrous Et_2O (20 mL) was added dropwise to a stirred solution of indole (3 g, 25.6 mmol) in Et_2O (20 mL) at 0 °C. After stirring the solution for 20 min, the bright yellow precipitate was removed by filtration and washed thoroughly with Et_2O. The crude product was added in portions to a solution of *N*-methylpiperazine (7.7 g, 76.8 mmol) in dry benzene (20 mL) and stirred for 16 h. The solvent was removed under reduced pressure to give a yellow semisolid material which crystallized after washing with water. Recrystallization from benzene gave 5.45 g (80%) of 31 as white flakes, mp 149–150 °C. Anal. ($C_{15}H_{17}N_3O_2$) C, H, N.

***N*^{1}-Methyl-*N*^{4}-[2-(3-indolyl)ethyl]piperazine (23).** A solution of 28 (1 g, 3.68 mmol) in dry THF (25 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (0.32 g, 8.4 mmol) in THF (25 mL) at 0 °C. The suspension was refluxed for 3 h, at which time $Na_2SO_4 \cdot 10H_2O$ was added portionwise until the evolution of H_2 ceased. The mixture was filtered, the filtrate was dried ($MgSO_4$), and the solvent was removed in vacuo to give a yellow oil which crystallized upon standing. Recrystallization from benzene–ligroin (bp 60–90 °C) gave 0.86 g (96%) of 23 as white crystals, mp 127–130 °C. Though previously reported as a HCl salt,[36] contact of 23 with HCl resulted in decomposition. The oxalate salt was prepared and recrystallized from 95% EtOH, mp 225–227 °C. Anal. ($C_{15}H_{21}N_3 \cdot 2C_2H_2O_6 \cdot 0.5H_2O$) C, H, N.

Binding Assay Studies. Sprague–Dawley rats, of either sex, weighing 200–250 g were used. The rat stomach fundus preparation employed was essentially that of Vane,[16] with the previously described modifications.[20] Two strips were cut from the same tissue and used in parallel 8-mL muscle baths. The relative sensitivity of the two strips was determined, after a 1-h equilibration period, by the use of 5-HT doses giving submaximal contractions. Only one compound was tested per preparation, the second strip serving as control.

The ability or potency of each agent to inhibit the contractile response to 5-HT was determined by obtaining cumulative dose–response curves to 5-HT, first in the absence of the agent in question and, then, in the presence of increasing concentrations thereof. The ED_{50} of 5-HT was determined for each of these curves, and the apparent affinities were calculated as pA_2 values by the method of Arunlakshana and Schild.[37] In several cases, the dose–response curves obtained were subjected to probit analysis using the Fortran program ISOBOL, whereby the slopes of the common regression line of the response metameter on the logarithm of the dose and the 5-HT concentrations required for half-maximal contraction were calculated. Results, however, did not vary between the two methods.

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Aminoglycoside Antibiotics. 2. *N,N*-Dialkylkanamycins

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$N^{6'}$,$N^{3''}$-Dialkyl derivatives of kanamycins A and B were prepared regiospecifically from the parent antibiotics. Although the dimethyl and diethyl derivatives of kanamycin A were inactive in standard antibacterial assays, the dimethyl derivative of kanamycin B showed moderate activity, especially against various strains of *Pseudomonas aeruginosa*. A method for the selective dimethylation of the 3″-amino group of kanamycin A also was developed.

The preparation of *N*-alkyl derivatives of the kanamycins has provided compounds with useful antibacterial activity. Thus, the $N^{6'}$-methyl derivatives of kanamycins A and B were similar in potencies to the parent antibiotics but less susceptible to inactivation by strains of bacteria that elaborate aminoglycoside-6′-acetyltransferase.[1,2] The $N^{3''}$-methyl derivatives of kanamycins A and B also were comparable in antibacterial potencies to the parent compounds;[1,3] however, the methyl groups did not afford protection against inactivation by aminoglycoside 2″-nucleotidyltransferase.[1] Larger alkyl groups at either $N^{6'}$ or $N^{3''}$ produced inactive molecules, but a series of N^1,-N^3,$N^{3''}$-trialkyl derivatives of kanamycin A in which the alkyl groups were benzyl or cyclohexylmethyl had low potency.[1] A unique property of the latter compounds was their nearly equal potencies against such diverse bacteria as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

There are no reported examples of $N^{6'}$,$N^{3''}$-dialkylkanamycins. However, this type of compound should be of interest because the same structural feature occurs naturally in gentamicins C_1 and C_{2_b} (sagamycin). Since it appeared that suitable $N^{6'}$,$N^{3''}$-dialkylkanamycins would have appreciable antibacterial activity, we undertook an investigation of their preparation and properties. The main problem in preparing these compounds lies in the regiospecificity of the alkylation reactions. Some selectivity can be obtained for $N^{6'}$, since it is the only amino group on a primary carbon, but $N^{3''}$ is one of the less reactive nitrogens in the kanamycins. As described below, a variety of methods utilizing different blocking groups were developed for $N^{6'}$,$N^{3''}$-dialkylkanamycins.

The synthesis of $N^{6'}$,$N^{3''}$-dimethylkanamycin B (**16**) from kanamycin B (**5**) began with the formation of penta-*N*-carbobenzyloxy derivative **6**.[4] Treatment of **6** with sodium hydride in *N,N*-dimethylformamide gave selectively the bis(cyclic carbamate) **10**, in which both the 6′ and 3″-amino groups are involved in the cyclic carbamates. That the five-membered cyclic carbamates were the 2″,3″-O,N derivative rather than the isomeric 4″,3″ derivatives was shown by the following evidence. Treatment of **10** and **12** with 1,1-dimethoxycyclohexane and *p*-toluenesulfonic acid in dry *N,N*-dimethylformamide gave cyclohexylidene derivatives **21** and **22** in nearly quantitative yields. These cyclohexylidenes could be formed only from the 2″,3″-*O,N*-carbonyl derivatives. Catalytic hydrogenolysis of **10** gave compound **11**, which has only these two amino groups acylated. Lithium aluminum hydride smoothly converted **11** into $N^{6'}$,$N^{3''}$-dimethylkanamycin B (**16**). $N^{6'}$,$N^{3''}$-Dimethylkanamycin A (**17**) was prepared from kanamycin A (**7**) by a route parallel to the one just described. It involved intermediates **8**, **12** and **13**.

Preparation of $N^{6'}$,$N^{3''}$-diethylkanamycins requires a different approach than the one utilized for the corresponding methyl analogues. A convenient synthesis of $N^{6'}$,$N^{3''}$-diethylkanamycin A (**18**) was obtained by a route based on the two O → N acetyl migrations that occurred when hexa-*O*-acetyl-tetra-*N*-carbobenzyloxykanamycin A (**14**) was hydrogenolyzed. This type of migration had been reported previously for $O^{4'} \rightarrow N^{6'}$ in a kanamycin A derivative by Kawaguchi and co-workers.[5] The synthesis of **18** started with the treatment of **8** with acetic anhydride in pyridine, which gave cleanly a hexa-*O*-acetyl derivative. This derivative probably has structure **14** because it is known from many experiments that the 5-hydroxyl group of a kanamycin is the least reactive one. Catalytic hydrogenolysis of **14** gave a tri-*O*-acetyl-$N^{6'}$,$N^{3''}$-di-*N*-acetylkanamycin A as the result of the acetyl migrations. An additional *O*-acetyl group was lost during this reaction or the isolation procedure. Probably this was the 6″-*O*-

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