Quantitative Relationships Between Structure and Pharmacokinetic Parameters Using Molecular Connectivity Chi Indices I: Substituted 2-Sulfapyridines

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Abstract □ Quantitative relationships between the structure and pharmacokinetic parameters of several compounds have been derived using the molecular connectivity approach. The correlation coefficients of equations obtained using the Chi indices (χ) as predictor parameters were compared favorably with those obtained by the linear free energy approach, where physicochemical parameters have been used as predictor variables. High correlation coefficients (r) for the first-order elimination rate constant (r = 0.86), total body clearance (r = 0.89), and protein binding association constant (r = 0.78) for substituted 2-sulfapyridines were obtained. However, including the pK_a (indicative of electronic effects) of the compounds within the equation as a predictor variable caused an increase in the correlation coefficients.

Keyphrases □ Molecular connectivity—Chi indices, use in quantitative structure–pharmacokinetic relationships, substituted 2-sulfapyridines □ Pharmacokinetic parameters—quantitative relationship to structure, determination by molecular connectivity Chi indices, substituted 2-sulfapyridines □ Quantitative structure–pharmacokinetic relationships—application of molecular connectivity Chi indices, substituted 2-sulfapyridines

When considering the expense of synthesizing and testing compounds, it is economically advantageous to screen a few with high therapeutic potential rather than mass screening of a large number of compounds. With this goal in mind, medicinal chemists have applied quantitative structure activity relationships (QSAR) to drug design. In the free energy approach, Hansch (1) used physicochemical properties, *e.g.*, the partition coefficient and dissociation constant, as predictor variables in QSAR. These physicochemical properties were correlated with biological activity using regression analyses. The result of the treatment was an equation which describes, in a quantitative manner, the relationship between biological activity of a compound and its chemical structure.

An area which has been ignored until recently is the role of structural changes in altering pharmacokinetic parameters. It is known that alterations in the values of the pharmacokinetic parameters of a drug may result in critical changes in biological action (2). Recent successful work has been done deriving equations which relate various pharmacokinetic parameters of drug molecules to their corresponding physicochemical properties (3, 4). These derived equations, referred to as quantitative structure pharmacokinetic relationships (QSPR), offer promise for predicting pharmacokinetic parameters for compounds, given their physicochemical properties. In many studies, the physicochemical properties of the compounds must be determined experimentally. This limitation may be a serious disadvantage in using the free energy approach in deriving QSPR.

Kier and Hall (5, 6) have developed the methodology of molecular connectivity, which gives numerical values, Chi indices (χ), that are directly related to the molecular structure of the compound (5, 6). The χ indices of various molecules have been shown to have a high correlation with the corresponding boiling point, partition coefficient, and many other physical properties of the molecule (6, 7). Molecular connectivity indices are useful as input parameters in defining QSAR. QSAR of the antimicrobial activity of halogenated phenols, the hallucinogenic activity of amphetamines, the anesthetic activity of hydrocarbons, and various other biological activities have been derived (5, 6) using the molecular connectivity approach. It has been reported that the χ index compares favorably with other possible predictor variables in correlating structure to the duration of anesthesia of a series of barbiturates (8).

It would appear that molecular connectivity might be used in deriving QSPR. The objective of this study was to examine the potential ability of molecular connectivity to quantitatively relate pharmacokinetic parameters to structure. The main advantage of using molecular connectivity in QSPR studies, rather than the free energy approach, is that the χ indices can be calculated for compounds that have not yet been synthesized. Therefore, by using the molecular connectivity χ indices as the predictor variables, the investigator no longer needs to synthesize and determine the physicochemical properties of compounds with predicted undesirable pharmacokinetic characteristics.

Molecular connectivity χ values contain no information regarding electronic influences through bonds or across space (6). Electronic influences may be crucial in the correlation of structure and pharmacokinetic characteristics for certain series of compounds (4). An additional problem in using χ indices as predictor variables is the fact that molecular connectivity can not differentiate between stereoisomers (6). However, the number of therapeutic agents in which stereochemistry influences the pharmacokinetic parameters is minimal.

The free energy approach (Hansch) has been fairly successful in deriving QSPR, and one could refer to it as

Table I—Correlation Coefficients (r) of Equations Relating Structure to Pharmacokinetic Parameters for Various Compounds

Pharmacokinetic Parameter	r^g	r^h	r^i
Percent barbiturate absorbed[a]	0.98[j]	0.99	0.99
P.C.[e] of neutral compounds[b]	0.93[j]	0.90	0.98
P.C.[e] of acidic compounds[b]	0.90[j]	0.99	0.89
K_a of sulfonamides[c]	0.89[k]	0.88	0.99
Percent $\Sigma X_b{}^f$ of penicillins[d]	0.93[k]	0.96	0.99+

[a] Ref. 11. [b] Ref. 12. [c] Ref. 13. [d] Ref. 14. [e] Permeability coefficient. [f] Cumulative biliary excretion. [g] Correlation coefficient of equations in which the log of the pharmacokinetic parameters are correlated with physicochemical properties. [h] Correlation coefficient of equations in which the log of the pharmacokinetic parameters are correlated with χ indices. [i] Correlation coefficient in which the antilogs of pharmacokinetic parameters are correlated with χ indices. [j] Ref. 4. [k] Ref. 3.

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Table II—Correlation Coefficient (r) and the Sum of Residual Squared of Equations Relating Structure to Pharmacokinetic Parameters for Substituted 2-Sulfapyridines

Pharmacokinetic Parameter	R^b	S^{2a}	R^c	S^{2a}	R^d	S^2
K_{el}, h^{-1}	0.95[e]	0.29	0.83	11.09	0.86	0.22
CL_T, mL/min	0.96[e]	1.29	0.87	1.94	0.90	1.17
K_{assoc}, L/mol	0.85[e]	1.3×10^8	0.83	2.7×10^8	0.78	2.7×10^8
$t_{1/2}$, h	—	—	0.86	5.5×10^2	0.81	4.7×10^2
Vd, mL	—	—	0.88	1.1×10^4	0.87	1.2×10^4

[a] Using the antilog of the predicted values. [b] Values for equations in which the log of pharmacokinetic parameters are correlated with physicochemical properties. [c] Values for equations in which the log of the pharmacokinetic parameters are correlated with χ indices. [d] Values for the equations in which the antilog of pharmacokinetic parameters are correlated with χ indices. [e] Ref. 3.

Table III—Statistical Comparison of Equations With and Without pK_a for Substituted 2-Sulfapyridine[a] Compounds

Pharmacokinetic Parameter	χ Indices r	χ Indices + pK_a r
K_{el}, h^{-1}	0.86	0.92
$t_{1/2}$, h	0.81	0.89
CL_T, mL/min	0.90	0.91
Vd, mL	0.87	0.87
K_{assoc}, L/mol	0.78	0.92

[a] Ref. 3.

the "accepted method." We wished to compare the molecular connectivity approach, the "new method," to the "accepted method" in the ability to derive QSPR. Comparison between the two methods was based on the values of correlation coefficients of the corresponding derived equations.

A commonly encountered problem in studying QSAR is in choosing which computer-generated statistically significant equation best describes the relationship between structure and biological activity (7). Possessing the highest value for the correlation coefficient (r), does not ensure that the particular equation is the best equation. Adding an independent variable may slightly increase the r value without truly improving the practical predictor value of the equation. In general, the most practical equation is that which has a relatively large r value with a minimal number of predictor variables (7). A similar problem has been encountered in fitting data using linear pharmacokinetic models. A statistical method referred to as Akaike's Information Criteria (AIC) has been applied with success to indicate which pharmacokinetic model is more appropriate to describe the data (9). Additionally, Akaike (10) has indicated that this method should be applicable as a statistical identification procedure for prediction studies. Therefore, the Akaike statistical method was applied in this study to determine the equation that best describes the relationship of structure to pharmacokinetic parameters.

EXPERIMENTAL

Molecular connectivity χ values were calculated for the compounds listed in Tables I and II using the computer program written by Hall[1]. The pharmacokinetic parameters or the logarithm of the pharmacokinetic parameters were the dependent variables and the χ values were the independent predictor variables. The independent variables of the tested compounds were entered through stepwise inclusion into the multiple regression analysis subprogram of the Statistical Package for the Social Sciences (SPSS). Statistically significant equations, $p < 0.05$ according to the overall (F value) and DF, generated by the multiple regression program were compared using the AIC. The AIC was calculated according to $AIC = N \ln (S^2) + 2P$, where N is the number of experimental data points, P is the number of parameters, and S^2 is the residual sum of squares (8). The equation with the minimal value of AIC was considered as the best equation. The generated best equations and their associated statistical terms were provided in the output of the regression computer program. They are listed in Appendices I and II; terms are defined in Appendix III.

[1] Dr. L. H. Hall, Eastern Nazarene College, Quincy, Mass.

RESULTS AND DISCUSSION

The use of molecular connectivity χ indices as independent variables in equations defining QSPR compares favorably to those equations derived using physiochemical properties as independent variables, as shown in Tables I and II. The data indicate the potential of the molecular connectivity approach in establishing QSPR. The use of the logarithm of the pharmacokinetic parameters, rather than the use of the original values, did not result in a universal increase in the values of the correlation coefficients (Tables I and II). It was found in this study that the use of the logarithm of the pharmacokinetic parameters might give misleading results.

The mathematical transformation from normal values (antilog) to the log values is known to result in statistical compression of extreme points on both sides of the midpoint of the range. These extreme values are often of the most interest to the drug designer. The prediction capability of an equation can be measured by high r associated with a minimal S^2. We found in some cases, that the use of the logarithms of pharmacokinetic parameters caused an increase in r with an increase in S^2, when S^2 is calculated from the antilog of predicted values (Table II). Thus, the use of the log-dependent variable may result in equations with high r and low prediction capability.

The theoretical basis for the linear free energy approach dictates that the log dependent variable be used. The molecular connectivity approach, however, may use either the log or the antilog of the observed parameters in the regression equations. This added flexibility is an advantage of the molecular connectivity approach over the linear free energy approach in deriving QSPR.

It would seem reasonable that electronic effects would influence the pharmacokinetic parameters of compounds with ionizable groups. Molecular connectivity χ values do not include information concerning electronic effects (pK_a) (6). When the pK_a was included with χ indices as a possible predictor variable in the regression analysis of the antilog pharmacokinetic parameters of substituted 2-sulfapyridines, there was an improvement in r values for K_{el}, $t_{1/2}$, and K_{assoc}, while Vd was not affected (Table III).

It appears from this study that employing both χ indices and independent variables reflecting electronic effects may be useful in developing QSPR for compounds with ionizable groups. Kier has suggested that Hückel molecular orbital parameters or Hammett sigma values may be useful for this purpose (6).

In conclusion, the use of the molecular connectivity approach is a new method with a high applicability to QSPR studies. This method appears comparable with the linear free energy approach in predicting QSPR. Further work on the use of the molecular connectivity in QSPR studies for other series of compounds is presently in progress.

APPENDIX I

Equations of the pharmacokinetic parameters of substituted 2-sulfapyridines generated by multiregression analysis, where the χ indices were the independent variables, are given herein.

Antilog of the Pharmacokinetic Parameters

Total body clearance (CL_T) in mL/min:

$$CL_T = 1.18\chi_{PC5} - 2.03\chi_{PC4} + 1.05\chi_{PCV5} - 17.2\chi_{PCV4} + 51.4\chi_{CV5} + 13.14\chi_{CV3} + 7.84$$

$r = 0.90$; $SE = 0.31$; $F = 8.38$; $DF = 6, 12$ (Eq. 1)

Apparent volume of distribution (Vd) in L:

$$Vd = -145.64\chi_{PC5} - 15.70\chi_{C5} + 275.55\chi_{C3} - 986.92\chi_{CV5} + 429.06$$

$r = 0.87$; $SE = 28.8$; $F = 10.7$; $DF = 4, 14$ (Eq. 2)

Biological half life ($t_{1/2}$) in h:

$$t_{1/2} = -23.72\chi_{PC5} + 27.30\chi_{PC4} + 136.62\chi_{PCV4} - 592.4\chi_{CV5} - 85.09\chi_{CV3} - 31.04$$

$r = 0.81$; $SE = 6.01$; $F = 4.94$; $DF = 5, 13$ (Eq. 3)

Overall elimination rate constant (K_{el} in h^{-1}):

$$K_{el} = 0.66\chi_{PC5} - 0.93\chi_{PC4} - 2.89\chi_{PCV4} + 12.92\chi_{CV5} + 1.75\chi_{CV3} + 1.55$$

$r = 0.86$; $SE = 0.13$; $F = 7.53$; $DF = 5, 13$ (Eq. 4)

Affinity constant for protein binding (Kassoc) in L/mol:

$$K_{assoc} = 10^3(-55.51\chi_{t4} + 38.62\chi_{t3} + 33.65\chi_{t2} + 53.13\chi_{tV5} - 54.28\chi_{tV4} - 99.74)$$

$r = 0.78$; $SE = 4982$; $F = 3.48$; $DF = 5, 11$ (Eq. 5)

Log of the Pharmacokinetic Parameters.

$$\text{Log } CL_T = 1.22\chi_{PC5} - 1.76\chi_{PC4} - 10.34\chi_{PCV4} + 36.77\chi_{CV5} + 7.65\chi_{CV3} + 4.06$$

$r = 0.87$; $SE = 0.30$; $F = 8.00$; $DF = 5, 13$ (Eq. 6)

$$\text{Log } Vd = -0.36\chi_{PC5} - 0.48\chi_{PC4} + 1.37\chi_{C5} + 1.21\chi_{C3} - 3.09\chi_{CV5} + 2.92$$

$r = 0.88$; $SE = 0.08$; $F = 9.21$; $DF = 5, 13$ (Eq. 7)

$$\text{Log } t_{1/2} = -1.53\chi_{PC5} + 1.99\chi_{PC4} + 8.18\chi_{PCV4} - 35.46\chi_{CV5} - 5.19\chi_{CV3} - 2.36$$

$r = 0.86$; $SE = 0.31$; $F = 7.50$; $DF = 5, 13$ (Eq. 8)

$$\text{Log } K_{el} = 0.69\chi_{t5} + 0.86\chi_{t4} - 2.75\chi_{t3} + 0.34\chi_{t1} - 6.63\chi_{tV5} + 10.56\chi_{tV4} - 4.30\chi_{tV3} + 6.95$$

$r = 0.83$; $SE = 0.37$; $F = 3.60$; $DF = 7, 11$ (Eq. 9)

$$\text{Log } K_{assoc.} = 0.98\chi_{t5} - 3.47\chi_{t4} + 3.16\chi_{t2} - 0.01\chi_{tV4} + 4.60\chi_{tV3} - 3.72\chi_{tV2} - 3.54$$

$r = 0.83$; $SE = 0.24$; $F = 3.83$; $DF = 6, 10$ (Eq. 10)

Electronic Effect (pK_a) Included as Another Independent Parameter with the χ Indices.

$$CL_T = 0.20pK_a - 0.91\chi_{P5} + 2.17\chi_{P4} - 1.30\chi_{P2} + 3.24$$

$r = 0.91$; $SE = 0.28$; $F = 16.0$; $DF = 4, 14$ (Eq. 11)

$$Vd = -145.64\chi_{PC5} - 15.7\chi_{C5} + 275.55\chi_{C3} - 986.92\chi_{CV5} + 429.06$$

$r = 0.87$; $SE = 28.8$; $F = 10.68$; $DF = 3, 14$ (Eq. 12)

$$t_{1/2} = -4.82pK_a - 18.52\chi_{t5} + 13.90\chi_{t2} - 10.82\chi_{tV2} + 24.84$$

$r = 0.89$; $SE = 4.55$; $F = 12.92$; $DF = 4, 14$ (Eq. 13)

$$K_{el} = 0.10pK_a - 0.58\chi_{P5} + 0.99\chi_{P4} + 0.87\chi_{P3} - 1.00\chi_{P2} + 0.01$$

$r = 0.92$; $SE = 0.10$; $F = 13.5$; $DF = 5, 13$ (Eq. 14)

$$K_{assoc} = -10^2(38.99pK_a - 99.11\chi_{P5} + 44.96\chi_{P4} + 63.13\chi_{P3} - 15.76\chi_{P2} - 30.11\chi_{P0} - 69.70)$$

$r = 0.92$; $SE = 3359$; $F = 8.74$; $DF = 6, 10$ (Eq. 15)

APPENDIX II

Equations of the pharmacokinetic parameters of compounds listed in Table I, generated by the multiregression analysis, where the χ indices were the independent variables are presented herein.

Antilog of the Pharmacokinetic Parameters.

Percentage of barbiturates absorbed in the colon (%A):

$$\%A = -25.83\chi_{t5} + 34.97\chi_{t4} - 39.8\chi_{t3} + 44.65\chi_{t1} + 15.11\chi_{tV5} - 16.25\chi_{tV1} - 41.41$$ (Eq. 16)

$r = 0.99$; $SE = 2.19$; $F = 29.7$; $DF = 6, 2$

Permeability coefficients of neutral compounds ($P.C._{neutral}$):

$$P.C._{neutral} = -829.9\chi_{t4} + 2138\chi_{t1} - 1287\chi_{tV5} - 1271\chi_{tV1} - 418.6$$ (Eq. 17)

$r = 0.98$; $SE = 219.6$; $F = 45.6$; $DF = 4, 6$

Permeability coefficients of acidic compounds ($P.C._{acidic}$):

$$P.C._{acidic} = -1319\chi_{t5} + 1072\chi_{t4} + 905.1\chi_{t2} + 818.9\chi_{tV5} - 1148\chi_{tV3} + 704.8$$ (Eq. 18)

$r = 0.89$; $SE = 481.7$; $F = 5.22$; $DF = 5, 7$

Absorption rate of sulfonamides (K_a):

$$K_a = 0.06\chi_{t5} - 1.57\chi_{t4} + 2.18\chi_{t3} + 0.38\chi_{t1} - 0.22\chi_{tV5} + 4.08\chi_{tV3} - 6.41\chi_{tV2} + 1.19\chi_{tV1} + 1.77$$

$r = 0.99$; $SE = 0.10$; $F = 18.8$; $DF = 8, 3$ (Eq. 19)

Percent cumulative excreted amounts of penicillins in the bile (ΣX_b):

$$\Sigma X_b = 50.46\chi_{t4} - 64.59\chi_{t3} + 16.47\chi_{t2} - 30.52\chi_{t1} - 53.83\chi_{tV3} + 55.89\chi_{tV1} + 231.11$$

$r = 0.99$; $SE = 0.85$; $F = 183.1$; $DF = 6, 2$ (Eq. 20)

Log of the Pharmacokinetic Parameters

$$\text{Log } \%A = -0.29\chi_{t4} - 0.02\chi_{t3} - 0.20\chi_{t2} + 0.56\chi_{t1} + 0.57\chi_{tV4} - 0.48\chi_{tV3} - 0.02\chi_{tV1} + 0.85$$

$r = 0.99$; $SE = 0.01$; $F = 527.8$; $DF = 7, 1$ (Eq. 21)

$$\text{Log } P.C._{neutral} = -0.45\chi_{t5} + 0.50\chi_{t4} + 0.09\chi_{tV5} + 3.03$$

$r = 0.90$; $SE = 0.14$; $F = 12.4$; $DF = 3, 9$ (Eq. 22)

$$\text{Log } P.C._{acidic} = 2.30\chi_{PV5} - 2.37\chi_{PV4} + 0.20\chi_{PV0} + 3.07$$

$r = 0.99$; $SE = 0.04$; $F = 219.8$; $DF = 3, 7$ (Eq. 23)

$$\text{Log } K_a = 0.24\chi_{PC4} + 3.54\chi_{CV5} - 2.47\chi_{CV3} + 0.56$$

$r = 0.88$; $SE = 0.19$; $F = 9.15$; $DF = 3, 8$ (Eq. 24)

$$\text{Log } \Sigma X_b = -1.54\chi_{P5} + 1.16\chi_{P4} + 0.96\chi_{P3} - 1.19\chi_{P2} + 5.88$$

$r = 0.96$; $SE = 0.08$; $F = 12.03$; $DF = 4, 4$ (Eq. 25)

APPENDIX III: GLOSSARY

χ Molecular connectivity Chi index
DF Degrees of freedom
F Variance ratio
r Correlation coefficient
S^2 Residual sum squared
SE Standard error of the mean

Subscripts
- c Cluster subgraph
- p Path subgraph
- v Valence
- n An integer indicating the order of χ
- t Total sum of n-order χ indices

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Digitonin Derivatives of Low Toxicity: Potential Solubilizers for Lipophilic Compounds

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Abstract □ Digitoxin was modified by condensation with propylene oxide or with 1,4-butanediol diglycidyl ether in aqueous alkali, yielding products in which some of the CH_2OH groups of digitonin were converted to $CH_2OCH_2CHOHCH_3$ or to $CH_2OCH_2CHOHCH_2O(CH_2)_4O$-$CH_2CHOHCH_2OH$ groups, respectively. These modified digitonins were very soluble in water and chloroform and effectively solubilized lipophilic compounds into aqueous solutions; *e.g.*, 2 mg of vitamin A or 0.6 mg of cholecalciferol could be dissolved per 1 mL of 5% aqueous solutions of modified digitonins. Compared with the toxicity of digitonin (LD_{50} 4 mg/kg iv), the toxicity of modified digitonin was greatly reduced: doses of 500 mg/kg by intravenous infusion were not lethal for mice.

Keyphrases □ Digitonin—derivatives, potential use as solubilizers, lipophilic drugs, comparative toxicity □ Solubilizers—digitonin derivatives, potential use with lipophilic drugs □ Lipophilic drugs—potential use of digitonin derivatives as solubilizers, comparative toxicity

Solubilizing agents are widely used both in pharmacy and pharmacology, and there is a constant need for new compounds that have low toxicity and do not functionally interfere with biomolecules (1, 2). Saponins, which are steroid glycosides, have some of the desirable properties (3). Two saponins, tomatin and digitonin, are readily available. Commercial preparations of tomatin have reasonable purity but are expensive, whereas for digitonin, which is rather widely used, the situation is reversed (4). The commercial preparation of digitonin is an extract of saponins from seeds of *Digitalis purperea*, further purified by treatment with cholesterol, which precipitates digitonin and related compounds. Such preparations are described as containing digitonin (40%), digalonin (15%), desglucodigitonin (25%), gitonin (15%), tigonin (3%), and dig d′ (2%) (5). The content of digitonin in commercial preparations is given as 70–80% (6). In most applications, the mixture is used. Despite its complexity, this mixture has unique properties and has been used extensively in biochemical pharmacology (7–9). Digitonin, on the other hand, even in the pure state, has some undesirable properties—its solubility in water is low and variable depending on the sample used (7) and on whether the digitonin sample had been previously treated with solvents (10). Furthermore, digitonin forms a complex with cholesterol, a process which is bound to have toxic and denaturating effects. In this work the aim was to modify the digitonin mixture chemically to overcome these defects; attempts were also made to make the necessary chemical modifications simple and easy to perform even with large quantities of material.

EXPERIMENTAL

Synthesis of Digitonin Derivative 2—Digitonin[1] (1 g, 0.8 mmol) was suspended in a solution of sodium hydroxide (200 mg) in water (6.5 mL), and propylene oxide (1 mL, 15 mmol) was added. The mixture was then stirred at 60°C for 1 h and at room temperature overnight. The clear solution was then neutralized by hydrochloric acid and dialyzed for 1 d against distilled water. Dialysis tubing from regenerated cellulose[2] was used with a nominal molecular weight cutoff of 8000–12,000. Freeze-drying of the contents of dialysis tubing gave derivative 2 as a solid foam-like material (1 g).

The solubility of derivative 2 in water was ~21 g/100 mL at room temperature. This compound tended to occlude solvents and thus, elemental analysis could not be used to estimate the degree of substitution of derivative 2. Field-desorption mass spectrometry[3] was used for that purpose; analysis of the relative intensities of peaks (mass of digitonin derivative plus sodium ion) gave the following distribution at the molecular weight: unsubstituted (36%), monosubstituted (36%), disubstituted (19%), trisubstituted (6%), tetrasubstituted (2%), and pentasubstituted (1%). Thus, the average degree of substitution is ~1.4.

Synthesis of Digitonin Derivative 3—The same procedure as above using 1,4-butanediol diglycidyl ether[4] in place of propylene oxide yielded 6.9 g of derivative 3 from 8 g of digitonin. The solubility of derivative 3 in water at room temperature was 11 g/100 mL.

To estimate the degree of substitution in derivative 3, the compound was exhaustively methylated and then analyzed for the content of carbon and methoxy groups. Derivative 3 (1 g, ~1 mmol) was dissolved in dimethylformamide (10 mL), sodium hydride (0.4 g) was slowly added, and then the mixture was stirred at room temperature for 30 min. The viscous mixture was then cooled to 0°C, methyl iodide (4.5 g) was added in a dropwise manner, and stirring was continued for another 12 h. Methanol was added, and the mixture was dialyzed against water and freeze-dried. Product 4 was a white powder (0.92 g) which was hygroscopic; completion of methylation was established by absence of absorption in the region of the hydroxyl stretching vibration (3100–3600 cm^{-1}) in the IR spectrum[5]. Compound 4 was repeatedly dissolved in water and evaporated

[1] Sigma Chemical Co., St. Louis, Mo.
[2] A. H. Thomas Co., Philadelphia, Pa.
[3] NIHLB assembly, Bethesda, Md.
[4] Aldrich Chemical Co., Philadelphia, Pa.
[5] Beckman infrared spectrophotometer IR12.

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