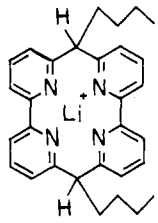

6

crown ethers, and the cavity size takes a very important part for complexation of metal ions.

Further studies toward additional structural analysis and variations of the macrocycle are now in progress.

Different Isotope Effects for Parallel Pathways of Enzyme-Catalyzed Transmethylation[1]

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Catechol O-methyltransferase[2] (EC 2.1.1.6, COMT) is one of the rare enzymes that catalyze parallel reactions of natural substrates, the methyl group of *S*-adenosylmethionine being simultaneously transferred to the *m*- and *p*-hydroxyl groups of dopamine, for example.[3] We now report that the kinetic isotope effects at pH 7.6 for the formation of these two products from *S*-adenosylmethionine labeled in the methyl group with ^3H and ^{14}C are quite different: $k_T/k_{14} = 1.16 \pm 0.07$ for meta methylation, $k_T/k_{14} = 1.35 + 0.05$ for para methylation. The value for para methylation agrees with an estimate of 1.29 ± 0.12 for S_N2 methyl transfer as purely rate determining. The smaller value for meta methylation, which is around 3-fold faster, indicates incursion of "physical steps" into determining the rate. A different transition-state structure for methyl transfer would also be in principle possible but is excluded by the further observation that at pH 6.2 both isotope effects become equal: 1.32 ± 0.10 (meta), 1.30 ± 0.06 (para).

The isotope effects were measured by allowing a mixture of *S*-adenosyl[*methyl*-^{3}H]methionine and *S*-adenosyl[*methyl*-^{14}C]-methionine (total concentration 0.03–0.05 mM) to methylate dopamine at 37 °C, pH 7.6, in Hepes buffer with 7.0 mM Mg^{2+}, with catalysis by rat-liver COMT. Dopamine was present in excess at concentrations of 0.25–7.5 mM. The meta and para products were isolated at various times by HPLC,[4] and the isotopic ratio $^3H/^{14}C$ was determined by liquid scintillation counting. The counts from the two isomeric products were pooled and the isotope ratio at various fractions of reaction was treated as usual[5] to obtain the isotope effect $k_T/k_{14} = J$. J is then a weighted average of effects for meta and para pathways (eq 1 and 2). To obtain the

$$J = W_m^{14} J_m + (1 - W_m^{14}) J_p \quad (1)$$

$$W_m^{14} = (m/p)_{14}/[1 + (m/p)_{14}] \quad (2)$$

individual isotope effects J_m and J_p, the separated meta and para products were counted, yielding $(m/p)_T = 2.77 \pm 0.05$; $(m/p)_{14} = 3.14 \pm 0.13$. Since it is also true that $J_m/J_p = (m/p)_T/(m/p)_{14}$, we can calculate J_m and J_p from the data.

J_m and J_p themselves vary[6] with dopamine concentration B, because the COMT mechanism is ordered with *S*-adenosylmethione binding first.[7] This binding is reversible at low B, allowing later steps to participate in limiting the rate, but becomes irreversible at high B. In Figure 1, J_m and J_p are extrapolated to $B = 0$ to obtain the *overall* isotope effects $J_{ovm} = 1.16 \pm 0.07$ and $J_{ovp} = 1.35 \pm 0.05$ and to $B = \infty$ to obtain a measure of the isotope effect for the "on reaction" of *S*-adenosylmethionine. The right-hand intercepts must be corrected for branching (see caption) but then yield the expected small or absent isotope effect for the binding step: $J_{on} = 1.03 \pm 0.03$ (meta); 1.02 ± 0.03 (para).

J_{ovm} and J_{ovp} can be compared to an expected value for fully rate-limiting S_N2 methyl transfer. This estimate can be made

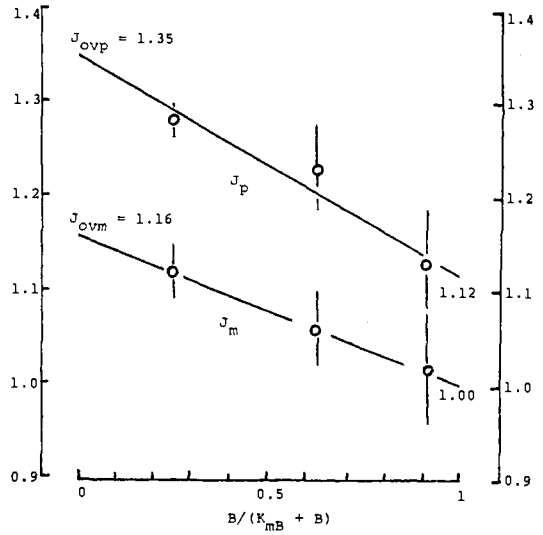

Figure 1. Plot of the observed isotope effects J_p (for para product) and J_m (for meta product), measured at various concentrations of dopamine (concentration = B) vs. a saturation function in B. The expected dependence of J on B (given by Northrop[6] in slightly different algebraic form) is:

$$J = (1 - F)J_{ov} + (F)J_{on}(\beta_T/\beta_{14})$$

where β_T and β_{14} are branching ratios between meta and para products, and $F = B/([K_{mB}k_2/k_5] + B)$ with k_2 measuring the off rate of *S*-adenosylmethionine from its binary complex with enzyme and k_5/K_{mB} the continuation rate of the binary complex on to products ($K_{mB} = 0.75$ mM).[3] The ratio β_T/β_{14} is 0.970 (meta) and 1.095 (para). In the figure, we have taken $k_2 \sim k_5$ so that $F = B/(K_{mB} + B)$; if this is correct, then the intercepts at $F = 1$ for J_m and J_p, when corrected by the branching ratios, will yield the same value of J_{on}. This is found, $J_{on} = 1.03 \pm 0.03$ (meta) and $J_{on} = 1.02 \pm 0.03$ (para), confirming that $k_2 \sim k_5$. The intercepts at $F = 0$ yield $J_{ovp} = 1.35 \pm 0.05$ and $J_{ovm} = 1.16 \pm 0.07$.

(1) This research was supported by the National Institutes of Health through research Grants GM-20199, GM-29332, and NS-10918 and by the National Science Foundation through the award of a Graduate Fellowship to W.P.H.

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from the directly measured[8] $k_{CH_3}/k_{CD_3} = 0.83 \pm 0.05$ and $k_{12}/k_{13} = 1.09 \pm 0.05$ for the maximal velocity of methylation of 3,4-dihydroxyacetophenone by COMT. The large magnitude of k_{12}/k_{13} here suggests that the S_N2 reaction is fully rate limiting.[8] Assuming equal contributions from each of the three deuteriums (rule of the geometric mean[9]) and the usual relations[10,11] between ^2H and ^3H and ^{13}C and ^{14}C isotope effects, we obtain $k_T/k_{14} = 1.29 \pm 0.12$. This is in good agreement with $J_{ovp} = 1.35 \pm 0.05$ suggesting that, for para methylation of dopamine, the S_N2 step alone determines the overall rate. For the meta pathway, $J_{ovm} = 1.16 \pm 0.07$, much smaller than expected for a pure S_N2 transition state, which indicates that a binding step or conformational change now "dilutes" the isotope effect. When the pH is lowered to 6.2, the S_N2 step slows in relation to the binding step. Then the S_N2 step determines the rate here also ($k_T/k_{14} = 1.32 \pm 0.10$).

Registry No. COMT, 9012-25-3; ^3H, 10028-17-8; ^{14}C, 14762-75-5; dopamine, 51-61-6.

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4,4′,4″-Tris(4,5-dichlorophthalimido)trityl: A New Type of Hydrazine-Labile Group as a Protecting Group of Primary Alcohols

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In the strategy for the synthesis of natural products containing both primary and secondary hydroxyl groups, the former has usually been protected with a trityl or hindered acyl group.[1] However, its selective removal required for further transformations is difficult, when acid- or base-sensitive functions are present in the same molecule. Recently, van Boom[2] has reported the use of hydrazine-labile levulinyl ester as a primary hydroxyl protecting group for oligonucleotide synthesis. However, this group lacks the selectivity in its introduction to primary alcohols of other substrates[3] and has inherent poor lipophilicity.

In this paper, we describe a new trityl-type of primary hydroxyl protecting group, 4,4′,4″-tris(4,5-dichlorophthalimido)trityl (CPTr), which is labile to hydrazine. We considered that 4,4′,4″-triphthalimidotrityl halides, which would be derived from tris(4-aminophenyl)methanol (pararosaniline) and phthalic anhydrides, might be used as tritylating agents to protect primary alcohols in the form of acid-stable trityl ethers owing to the strong inductive effect of the phthalimide groups and that upon hydra-

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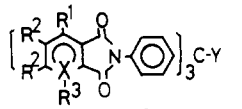
(2) (a) van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, 4875. (b) de Rooij, J. F. M.; Eille-Hazelegar, G.; van Deursen, P. H.; Serdijn, J.; J.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 537. (c) den Hartog, J. A. J.; van Boom, J. H. *Ibid.* **1981**, *100* 275. (d) den Hartog, J. A. J.; Wille, G.; van Boom, J. H. *Ibid.* **1981**, *100*, 320.
(3) The levulinations of 2′-*O*-(tetrahydropyran-2-yl)uridine and 2′-*O*-(1,3-benzodithiol-2-yl)uridine[4] by van Boom's method[2d] resulted in the 5′-levulinated products in 50–65% yields. This relatively poor selectivity may be due to the sterically less hindered 2′-*O*-protecting group relative to the 4-methoxy(tetrahydropyran-4-yl) group that has a quaternary carbon bound to the 2′-oxygen.

Chart I



	R^1	R^2	R^3	X	Y
1a:	H	H	H	C	OH
1b:	H	Cl	H	C	OH
1c:	Cl	Cl	H	C	OH
1d:	H	H	–	N	OH
CPTrBr:	H	Cl	H	C	Br

Scheme I

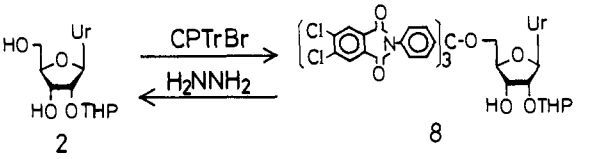
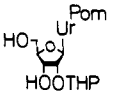
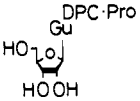
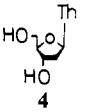
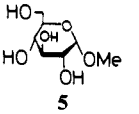
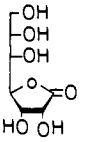
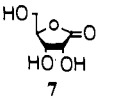


Table I. Results and Conditions of the Reactions of **2–7** with CPTrBr[a,b]

substrate compd	2,6-lutidine, equiv	time, min	primary CPTr ether yield, compds	%
 **2**	2[c]	15	8	84
 **3**	0	15	9	73
 **4**	2	30	10	85
 **5**	2	20	11	75
 **6**	2	20	12	88
 **7**	2	20	13	70

[a] These reactions were carried out at room temperature by using 2 equiv each of CPTrBr and $AgNO_3$ in dimethylformamide (10 mL/(L mmol of the substrate)). [b] In the case of compounds containing an acid-sensitive group, 2,6-lutidine was added prior to addition of CPTrBr. [c] When 2,6-lutidine was eliminated, the THP group was lost to a considerable extent (~15%).

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