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Thiolate Alkylation in Tripod Zinc Complexes: A Comparative Kinetic Study

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The biologically relevant alkylations of the thiolate ligands in tripod zinc thiolates by methyl iodide were studied kinetically. Five tripod ligands of the pyrazolyl/thioimidazolyl borate type were employed, offering N_3, N_2S, NS_2, and S_3 donor sets. For each of them, the ethyl-, benzyl-, phenyl-, and *p*-nitrophenylthiolate zinc complexes were investigated, yielding a total of 20 second-order rate constants. The comparison of these rate constants shows three effects: (1) the electronic effect among the thiolates, i.e., the ethanethiolates react about 3 orders of magnitude faster than the *p*-nitrophenylthiolates; (2) the steric effect among the pyrazolylborates, i.e., the phenyl-substituted ones react about 2 orders of magnitude faster than the *tert*-butyl-substituted ones; and (3) the strong acceleration by the sulfur donors in the tripods, reaching 4 orders of magnitude between the reaction times of the (N_3)Zn–SR and (S_3)Zn–SR complexes.

Introduction

Biological thiolate alkylation is one of the more-recent additions to the long list of biological functions for which zinc enzymes are active,[1,2] and the easy availability of zinc thiolate complexes has induced several inorganic chemists, including ourselves, to model the enzymatic process with such complexes. This is the fifth paper in our series of investigations on this subject and concludes our studies. In our previous publications,[3−6] we have outlined our approach and given ample reference to the valuable contributions of all our competitors, which are also cited in Parkin's recent review.[2]

Our major contribution to the modeling of biological thiolate alkylations has been the synthesis and chemical investigation of a complete series of tripod zinc thiolate complexes in which the pyrazolylborate-derived tripods possess N_3, N_2S, NS_2, and S_3 donor sets.[3−6] These tripods offer pyrazole nitrogen (in place of the enzymes' histidine) and thioimidazole sulfur (in place of the enzymes' cysteinate) as the donors for zinc. Although this is only a rough representation of the biological situation, it is the best one so far, both with respect to the reliability of the permanent attachment of the tripods to zinc through the whole course of complex interconversions and with respect to clean and quantitative thiolate alkylations.

There is reasonable agreement that the alkylation reactions are intracomplex, i.e., they occur at the zinc-bound thiolates, both in the enzymes and in the model complexes.[1,2] However, there is evidence that in a sulfur-only environment of zinc, for instance in $Zn(SR)_4^{2-}$ complexes, this is not so and that the thiolate dissociates from zinc before alkylation.[7,8] In our own preliminary kinetic studies, we always observed clean second-order reactions between the tripod zinc thiolates and methyl iodide.[3−6] We found, however, that there are considerable differences in reactivity between zinc thiolate complexes of the different tripod ligands. Having made available the tripod zinc thiolates for the full range of N/S

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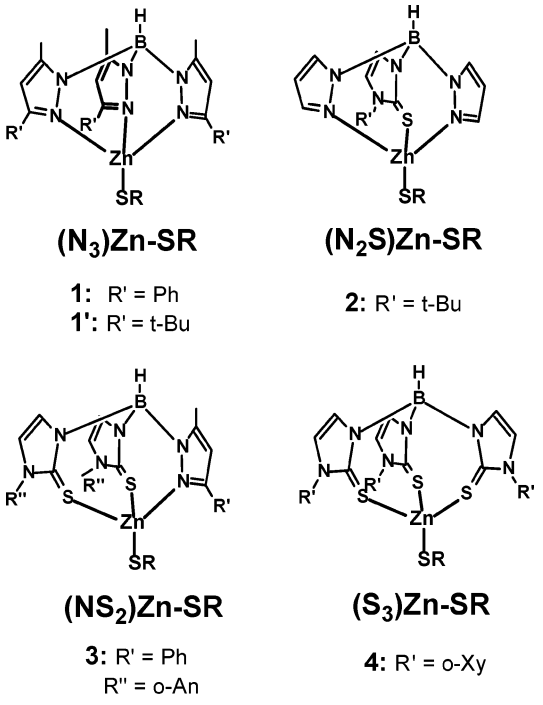
Scheme 1



donor sets and a complete array of thiolates, we had in our hands the material for a comprehensive kinetic study.

The present paper reports this kinetic study. It was undertaken to elucidate the factors that affect the rate of alkylation by varying the donor sets of the tripods, the nature of the thiolates, and in one instance, the steric requirements of the tripod. We hoped to gain some insight as to why nature prefers sulfur-rich (typically NS_2Zn) environments to anchor zinc in the enzymes and possibly learn how the inorganic model complexes might be modified in order to become as efficient as the enzymes in the alkylation reaction.

Results and Discussion

Materials and Methods. Scheme 1 lists the ligands employed and their zinc thiolate complexes used for this study. The choice of the specific tripods was dictated by the availability of all four thiolate complexes for each of them. This explains the somewhat irregular variation of the substituents on both the pyrazole and thioimidazole rings along the series. To get unambiguous information on a steric effect, we studied two series of tris(pyrazolyl)borate complexes: one with phenyl and the other with *tert*-butyl substituents at the 3-positions of the pyrazole rings. The four thiolates were each chosen to span the whole range of electron densities at sulfur. All 20 complexes used have been described by us before, as have most of their reactions with methyl iodide according to eq 1.

$$L\cdot Zn{-}SR + Me{-}I \rightarrow L\cdot Zn{-}I + MeSR \qquad (1)$$

The kinetic measurements were performed in chloroform solutions. The temperature was 300.0 K, except for **3a** and **4a**, which reacted too quickly and decomposed slowly at this temperature. Pseudo-first-order conditions were applied by using methyl iodide in a 5−20-fold excess. The reactions were followed by ^{1}H NMR, recording the intensities of characteristic singlet resonances, e.g., the methyl signal of MeSR or the methylene signal of the benzyl-containing species. The second-order rate constants were obtained from the plots of the pseudo-first-order rate constants versus the Me−I concentration. The k″ values for **1b**,[3] **2b**,[4] **3d**,[5] and **4d**[6] have been reported by us already. As graphic representations of the kinetic measurements and of their evaluation have been given in all those four cases,[3−6] no further ones are shown here and only their numerical results are dealt with (see Experimental Section).

Data. The results of the measurements are listed in Table 1. The k″ values are given for 300 K, which means that the data for **4a** and **4b** are extrapolated (see Experimental Section). It is evident that a very large range of rate constants is observed, with almost 7 orders of magnitude between the fastest (**4a**) and the slowest (**1′d**) reaction. The widest range within the same tripod is that for **2** (N_2S), with 4 orders of magnitude. The widest range within the same thiolate is that for the *p*-nitrothiophenolate, also with 4 orders of magnitude.

Influence of the Thiolates. Figure 1 shows a logarithmic display of the rate constants, to visualize the influence of the thiolates. There is no crossover of the lines, which means that in all five complex types, the order of reactivities of the thiolates is the same. Furthermore, the sequence SEt > SBn > SPh > SNit corresponds to expectations. On average, the S-ethyl complexes react half an order of magnitude faster than the S-benzyl complexes. These in turn react about 1 order of magnitude faster than the S-phenyl complexes, which then react about 2 orders of magnitude faster than the S-*p*-nitrophenyl complexes.

Among the many definitions and tabulations of relative nucleophilicities in the chemical literature, there seems to be none which compares the different thiolates. In the extensive tables compiled by Pearson,[9] only hydrosulfide, thiophenolate, and sulfite are listed, being among the fastest substrates for methylation by methyl iodide. Thus, the data presented here seem to provide some basic information in this respect.

Influence of the Tripods. Figure 2 displays the data set such as to visualize the effects of the tripod ligands. Again, the appearance is rather homogeneous, with no crossover of the curves and a general difference of about 4 orders of magnitude between the fastest (S_3) and the slowest (N_3(*t*-Bu)) systems. Were it not for the N_3(Ph) system, there would be a clean progression of rates in the sequence $N_3 < N_2S < NS_2 < S_3$. Furthermore, the graph suggests a ranking of the systems into three groups: slow (N_3(*t*-Bu)), medium (N_2S and N_3(Ph)), and fast (NS_2 and S_3).

Evaluation. Thiolates. The bonding situation of the thiolate ligands in these complexes is somewhere intermedi-

(9) Pearson, R. G.; Sobel, H.; Songstad, J. *J. Am. Chem. Soc.* **1968**, *90*, 319.

Table 1. Second-Order Rate Constants (M^{-1} s^{-1}) at 300 K

	N_3(Ph)	N_3(*t*-Bu)	N_2S	NS_2	S_3
SEt	**1a**: 5.0×10^{-2}	**1′a**: 1.6×10^{-4}	**2a**: 1.5×10^{-2}	**3a**: 2.7×10^{-1}	**4a**: 7.8×10^{-1}
SBn	**1b**: 1.8×10^{-2}	**1′b**: 7.6×10^{-5}	**2b**: 6.5×10^{-3}	**3b**: 9.8×10^{-2}	**4b**: 3.7×10^{-1}
SPh	**1c**: 2.0×10^{-3}	**1′c**: 1.0×10^{-5}	**2c**: 1.4×10^{-4}	**3c**: 6.2×10^{-2}	**4c**: 1.1×10^{-1}
SNit	**1d**: 2.0×10^{-5}	**1′d**: 3.3×10^{-7}	**2d**: 2.0×10^{-6}	**3d**: 1.5×10^{-3}	**4d**: 2.2×10^{-3}

ate between purely ionic (such as in the free thiolates) and purely covalent (such as in the thioethers). One would therefore like to compare their reactivities with those of these two reference systems. This, however, is hampered by the lack of data on the side of the reference systems. Pearson's tabulations[9] reveal the anionic sulfur compounds to be the strongest nucleophiles toward methyl iodide and they show that these compounds react about 5 orders of magnitude faster than the thioethers. Yet, even under conditions (methanol, 25 °C) that are not too different from those employed here (chloroform, 27 °C), the thioethers react faster than all (except the very fastest) of the thiolate complexes. This, of course, points to the steric hindrance provided by the encapsulating tripod ligands and is therefore another indicator that the alkylation reactions occur at the zinc-bound thiolates. But it also prevents these data from yielding an answer to the question of how anionic the thiolate ligands are in these tripod zinc complexes.

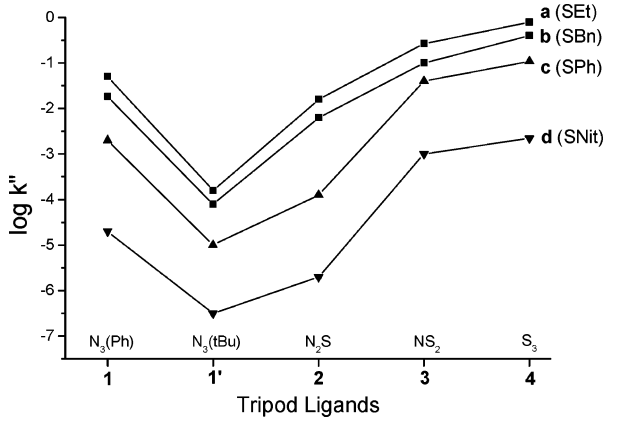


Figure 1. Logarithmic plot of the reaction rates for the four different thiolates as a function of the tripod zinc units. Abbreviations: Et = ethyl, Bn = benzyl, Ph = phenyl, Nit = *p*-nitrophenyl.

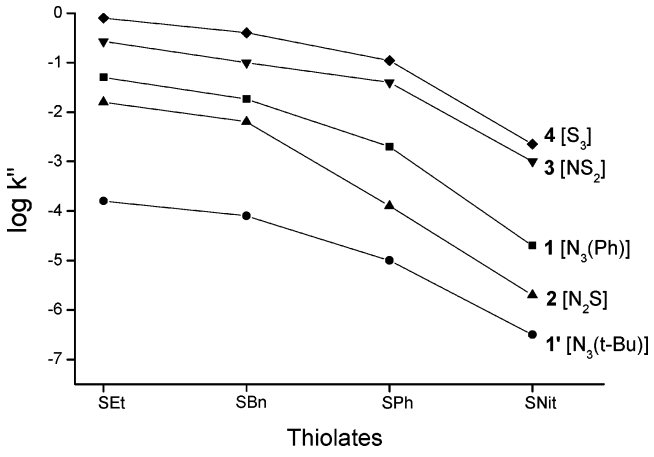


Figure 2. Logarithmic plot of the reaction rates for the five different tripod zinc units as a function of the thiolates. Abbreviations: Et = ethyl, Bn = benzyl, Ph = phenyl, Nit = *p*-nitrophenyl.

In comparison to the biological thiolate alkylations,[1,2] neither of the complexes investigated here is as efficient as the zinc enzymes. Although the enzymatic environment of the zinc ions should be at least as crowded as that in the complexes, nature has found ways to activate the thiolate ligands in this environment. Hydrogen-bonding patterns are often proposed to explain such phenomena. It should be pointed out, however, that in related complexes, hydrogen bonding involving the thiolate ligands has been found to retard the alkylation.[8,10,11] We therefore propose that the enzymatic efficiency rests mainly in a design of the enzymes that increases the anionic nature of the zinc-bound thiolate substrates.

Tripods. As mentioned already, the five tripod zinc units can be put into three groups in terms of reactivity. Steric hindrance can easily explain that the N_3(*t*-Bu) system is the slowest to be methylated, with many structure determinations having shown how well the $Tp^{t-Bu,Me}$ ligand encapsulates various Zn−X units.[2] On the other hand, the $Tp^{Ph,Me}$ ligand leaves considerably more space for the Zn−X units, regardless of the fact that it creates a larger hydrophobic cavity. Thus, the comparison of the methylation rates of the N_3(Ph) and N_3(*t*-Bu) systems yields a quantitative measure of a steric effect, which amounts to 2−2.5 orders of magnitude here.

Although it is easy to understand that the [N_3(*t*-Bu)]Zn−SR complexes are the slowest to react, it is quite difficult to understand why the [N_2S]Zn−SR complexes are grouped with, but are slower than, the [N_3(Ph)]Zn−SR complexes. One would expect them to be faster; first, because there is the general trend of increasing rate with increasing number of sulfur donors, and second, because the N_2S ligand used provides the least steric hindrance of all ligands employed here. We offer the tentative explanation that there is a favorable electronic effect due to the hydrophobic cavity around the Zn−SR unit created by the three phenyl substituents of the $Tp^{Ph,Me}$ ligand. Whatever this effect may be, it reverses the "natural" order of reaction rates, placing the line of the [N_3(Ph)]Zn−SR complexes in Figure 2 above the line for the [N_2S]Zn−SR complexes.

The third and most-reactive group is that of the [NS_2]Zn−SR and [S_3]Zn−SR complexes. They are the ones that establish the natural order of reactivities: $N_3 < N_2S < NS_2 < S_3$. One would have expected this, both in relation to nature's preference for [NS_2]Zn and [S_3]Zn enzymes to perform thiolate alkylation[1,2] and because one would intuitively assume a soft reaction (thiolate + methyl iodide) to be favored in a soft environment.

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Table 2. Data of the Kinetic Measurements

complex, concentration (M)	CH_3I concentration (M), k_{obs} value (s^{-1})					k_{obs} multiplier
1a, 0.011	0.055, 2.56	0.076, 3.61	0.098, 5.02	0.120, 5.60	0.140, 7.00	$\times 10^{-3}$
1b, 0.020	0.10, 1.78	0.12, 1.96	0.14, 2.62	0.16, 2.84	0.18, 3.08	$\times 10^{-3}$
1c, 0.014	0.068, 2.71	0.14, 3.58	0.20, 4.93	0.27, 6.67	0.34, 7.98	$\times 10^{-4}$
1d, 0.012	0.12, 0.84	0.18, 2.19	0.24, 3.33	0.30, 3.58	0.36, 5.95	$\times 10^{-6}$
1′a, 0.012	0.12, 0.98	0.18, 2.02	0.24, 2.97	0.30, 4.03	0.36, 4.99	$\times 10^{-5}$
1′b, 0.010	0.054, 0.39	0.109, 0.71	0.163, 1.00	0.218, 1.40	0.272, 2.00	$\times 10^{-5}$
1′c, 0.011	0.167, 2.56	0.223, 3.20	0.279, 3.79	0.334, 4.55	0.390, 5.18	$\times 10^{-6}$
1′d, 0.011	0.222, 6.10	0.444, 6.73	0.555, 7.21			$\times 10^{-7}$
2a, 0.016	0.16, 1.81	0.19, 2.06	0.22, 2.60	0.25, 3.18	0.28, 3.66	$\times 10^{-3}$
2b, 0.014	0.11, 4.92	0.14, 7.28	0.16, 8.93	0.19, 10.1	0.22, 11.7	$\times 10^{-4}$
2c, 0.0038	0.115, 3.37	0.134, 5.40	0.153, 7.63	0.172, 10.85	0.191, 14.06	$\times 10^{-6}$
2d, 0.0096	0.24, 0.90	0.29, 1.71	0.34, 2.43	0.38, 3.68	0.43, 4.60	$\times 10^{-7}$
3a, 0.010	0.050, 1.84	0.065, 6.51	0.080, 9.79			$\times 10^{-3}$
3b, 0.006	0.050, 0.68	0.080, 3.39	0.100, 5.28			$\times 10^{-7}$
3c, 0.006	0.033, 2.63	0.046, 3.40	0.059, 4.15	0.073, 5.25	0.086, 5.78	$\times 10^{-3}$
3d, 0.010	0.17, 2.32	0.21, 2.88	0.25, 3.70	0.29, 4.21	0.34, 4.94	$\times 10^{-4}$
4a,[a] 0.010	0.050, 1.4	0.075, 3.9	0.100, 5.7			$\times 10^{-3}$
4b,[a] 0.010	0.050, 0.47	0.075, 1.54	0.100, 2.33			$\times 10^{-3}$
4c, 0.010	0.050, 0.60	0.075, 3.91	0.100, 5.45	0.125, 8.12	0.150, 11.6	$\times 10^{-3}$
4d, 0.010	0.10, 1.08	0.15, 1.67	0.20, 3.91	0.25, 4.19	0.30, 5.40	$\times 10^{-4}$

[a] Measured at 280 K.

Searching for a quantitative correlation between the observed reactivity sequence and another physical quantity of these complexes, we became aware of such a quantity in the form of the Zn−S(thiolate) bond lengths. As mentioned before,[6] these grow steadily in the same sequence as the reaction rates. The example of the Zn−SEt complexes most clearly underlines this: their Zn−S(thiolate) bond lengths are 2.20 Å for the N_3 system,[12] 2.23 Å for the N_2S system,[4] and 2.27 Å for the S_3 system.[6] These data seem to indicate an increase in the electron density at zinc with an increasing sulfur content of the tripod ligands, which is translated into a more-ionic nature of the thiolate ligands. The ionic nature, in turn, manifests itself in the reactivity toward alkylation. In line with this is the observation that the sulfur-rich zinc thiolate complexes can dissociate to yield free anionic thiolate prior to alkylation.[7,8]

Mechanistic Alternatives. Following this line of thought leads to the argument that it may be the ease of thiolate dissociation from the complexes that determines the rate of alkylation. As discussed before,[7,8] this may lead to the same S_N2-like rate behavior, and independent measurements would be necessary to unambiguously eliminate this alternative. Such measurements have been performed by Parkin for the (S_3)Zn−thiolate system,[8] indicating a minute, but not convincing, preference for thiolate dissociation. We investigated thiolate-exchange reactions for the (N_3)Zn−SR and (S_3)Zn−SR systems[3,6] under the same conditions as those used for the alkylation reactions. They were much slower for the (N_3)-Zn−SR complexes and about as fast as the alkylations for the (S_3)Zn−SR complexes, in line with the statements made in the Introduction that in all cases but the (S_3)Zn−thiolate systems, the alkylations occur at the zinc-bound thiolates. The strong steric effect observed for the two different (N_3)-Zn−SR systems above supports this, and hence we assume this mechanism to be the correct one.

Another mechanistic alternative would involve the temporary dissociation of a ligand arm (i.e., one of the tripod donors or one of the amino acid side chains in the enzyme) during the alkylation process. The kinetic implications of this would be difficult to assess, as it would be difficult to design experiments proving or disproving it. However, circumstantial evidence speaks against this alternative. First, it would reduce the coordination number of zinc to three (dissociative ligand exchange at a tetrahedral complex), which is somewhat unlikely. Second, among the several hundred molecular structures of these tripod-Zn−X complexes, there are less than a handful of those in which the tripod is not tridentate and none in which the zinc ion is three-coordinate. Thus we do not consider this alternative to be a viable one.

General Considerations. With respect to the two motivations for this work mentioned in the Introduction, there seems to be a reasonable conclusion for the one concerning nature's choice of the donor environment of zinc in the enzymes. As this work has shown, the sulfur-rich tripods produce the most-reactive zinc thiolate complexes. With the additional observation that the (NS_2)Zn−SR complexes are not much slower than the (S_3)Zn−SR complexes and that the latter belong to the group that tends to dissociate and liberate anionic thiolate, one can understand that the (NS_2)Zn system is optimal for its purpose, namely the alkylation of the thiolate in the zinc-bound state.

Concerning the question of how one might optimize the tripod ligands such that their reactivity reaches that of the enzymes, the answer seems to be more difficult. Among our tripods, the maximum seems to be reached in the S_3 tripods, and the reaction rates of their Zn−SR complexes may at best be raised by 1 order of magnitude using suitable substituents. But that would improve them only to such a degree that they react at reasonable rates with trimethyl phosphate,[6] whereas no L·Zn−SR complex has been found yet that can be alkylated with natural reagents, e.g., alkylammonium salts such as methyl tetrahydrofolate.[1] Although it is difficult to compare our rate constants with those of other related complexes (other solvents, other temperatures, other thiolates),[2,7,8,10,11] it is a fact that no tripod-zinc−thiolate

(12) Alsfasser, R.; Powell, A. K.; Trofimenko, S.; Vahrenkamp, H. *Chem. Ber.* **1993**, *126*, 685.

complex is nearly as reactive as the enzymes. It therefore seems that an improvement in the tripod ligands must go along with a better understanding of how the enzymatic environment of the zinc ions tunes their Zn−SR centers for speed of alkylation. As the large difference between the N_3(*t*-Bu) and N_3(Ph) systems shows, the substituents on the ligands will be the decisive factors of this improvement.

Experimental Section

All experiments were carried out in a nitrogen atmosphere. All thiolate complexes were prepared as described.[3−6]

All solutions, glassware, and the probe chamber of the ^{1}H NMR spectrometer were thermostated to 300.0 K. All reagents were applied as standard solutions in $CDCl_3$ (99.99%) and were stored in the dark. The reagents were combined immediately prior to the measurements. The concentrations of the thiolate complexes were adjusted to 0.01−0.02 M; 3−5 kinetic runs were performed for each complex with methyl iodide concentrations in a 5−20-fold excess. The intensities of one or two well-isolated ^{1}H NMR resonances were recorded automatically for five $t_{1/2}$ times and stored for digital data processing, which yielded the pseudo-first-order rate constants. Each kinetic run was repeated at least once, and the data were reproducible within 10%. The plots of the resulting k_{obs} values against the corresponding CH_3I concentrations yielded the second-order rate constants with correlation coefficients >0.99. The kinetic runs with **4a** and **4b** were performed at 280 K, yielding k″ values of 8.7 and 3.7×10^{-2} M^{-1} s^{-1}. Using the empirical formula $k'(T + 10)/k(T) = 3$[13] as a rule of thumb, we converted the values to the 300 K values listed in Table 1. Table 2 lists the experimental data.

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(13) See textbooks of physical chemistry.