Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 4295–4298

TETRAHEDRON LETTERS

Amplification of diastereoselectivity by cyclodextrins in the copper-mediated cleavages of methylphosphonamidothioates

Robert A. Moss* and Jingzhi Tian

Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903, USA

Received 5 March 2003; revised 17 March 2003; accepted 17 March 2003

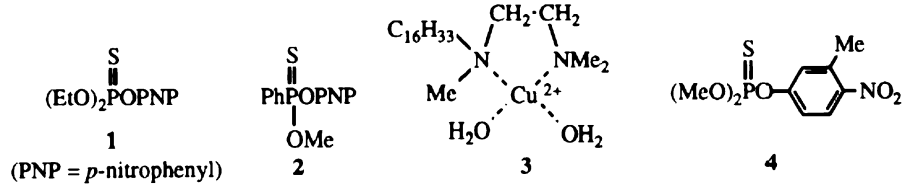
Abstract—Cu-mediated cleavage, coupled with diastereoselective binding and orientational preferences supplied by γ-cyclodextrin, lead to substantial kinetic diastereoselectivity between phosphonamidothioate diastereomers.

Continuing interest in the degradation of phosphonate and phosphonothioate toxins has stimulated much research focused on model substrates or 'simulants.'[1] We have examined the hydrolyses of the thiophosphate and phosphonothioate substrates parathion (**1**) and *O*-methyl *O*-4-nitrophenyl phenylphosphonothioate (methyl-EPN) (**2**) mediated by several reagents, including the copper 'metallomicelle' derived from *N*-*n*-hexadecyl-*N*,*N*′,*N*′-trimethylethylenediamine, **3**.[2] In the case of chiral **2**, for example, we found that Cu–OH hydrolytic cleavage mediated by micellar **3** occurred with complete inversion at phosphorus[2b] and with substantial acceleration.[2a,b]

Cyclodextrins are among the preeminent models for hydrolytic enzymes; their ability to recognize, bind, and catalyze the cleavage of complementary substrates has been extensively documented.[3] However, their reactivity toward phosphorus ester substrates has been only sparsely studied. α-Cyclodextrin (α-CD) is reported to stereoselectively cleave the enantiomers of isopropyl methylfluorophosphonate (sarin),[4] and a covalent *o*-iodosobenzoate-β-CD conjugate binds and cleaves the nerve agent soman at its P–F bond.[5] Phosphonates,[6a] phosphates,[6b] and phosphodiesters[6c] have also been examined. Our laboratory described the β-CD and γ-CD catalyzed hydrolyses of several *p*-nitrophenyl phosphotriesters, including the activated *p*-nitrophenyl 1,8-naphthyl phosphate. Here, as in other phosphorolyses mediated by simple CD catalysts, rate accelerations were relatively low (<100).[7]

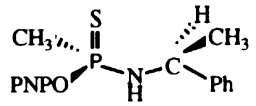
Recently, two important new studies of CD-mediated phosphate[8] and thiophosphate[9] reactions have appeared: Easton et al. reported that Cu complexes of diaminoalkyl-β-CD derivatives afforded 7–9.5×10^4 rate accelerations in the pH 7 hydrolyses of 4-*t*-butyl-2-nitrophenyl dimethyl phosphate,[8] whereas de Rossi et al. observed that basic hydrolysis of the thiophosphate insecticide fenitrothion (**4**) was *inhibited* by native β-CD, which bound **4** so that its labile thiophosphate linkage was shielded from external OH^-, and not suitably oriented for attack by the (*sec*) OH(O^-) groups of the CD.[9]

Here we report that CD-inhibition of thiophosphonate hydrolysis can be harnessed to effect strong amplification of stereoselectivity in cleavages of the diastereomeric phosphonamidothioates (S_PS_C)-**5** and (R_PS_C)-**5**. As these compounds can be used in stereospecific syntheses of phosphonothioate enantiomers,[2b,10] their stereoselective hydrolyses could, in principle, be incorporated into enantioselective syntheses based on kinetic resolutions.

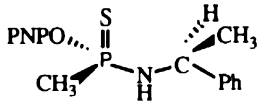


* Corresponding author.

0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)00782-2



(S_PS_C)-**5**
(mp 79-80 °C, $[\alpha]_D^{25}$+61.0°)



(R_PS_C)-**5**
(mp 120-120.5 °C, $[\alpha]_D^{25}$-121.2°)

The diastereomeric phosphonamidothioates were prepared from racemic 4-nitrophenyl methylphosphonothiochloridate [MeP(=S)ClOPNP] and (*S*)-(−)-α-phenylethylamine as described by Reddy and Kovach.[10] The diastereomers were separated and purified by fractional crystallization[10] and their mp's, ^{31}P NMR resonances, and optical rotations were in good agreement with reported values.[10,11] The diastereomer with mp 79–80°C and $[\alpha]_D^{25}$ +61.0° was assigned the (S_PS_C) configuration based on a single crystal X-ray structure determination.[12]

Diastereomeric substrates **5** were subjected to Cu-mediated cleavage in the presence or absence of α, β, or γ-CD. The Cu reagents included the 1,2-diaminocyclohexane derivatives (1*R*,2*R*)-**6**,[13] (1*R*,2*R*)- and *rac*-**7**,[14,15] Cu^{2+}–kanamycin A (**8**),[16] and $Cu(NO_3)_2$.

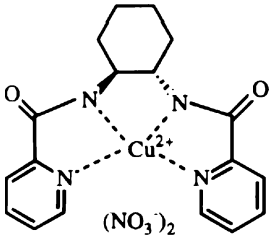
Cleavage rates were initially measured in the absence of CD, but in the presence of 5-fold excess Cu catalyst at pH 7 in HEPES buffer. The release of *p*-nitrophenoxide was followed at 400 nm by UV spectroscopy. The first section of Table 1 collects the observed rate constants for the Cu-mediated cleavages of (*SS*)-**5** and (*RS*)-**5**.

The Cu reagents greatly accelerate the cleavages of substrates **5**: in the absence of Cu, reactions failed to occur over 40 h. Based on a half-life of ~8 min for the 'slow' reaction of **6** with (*RS*)-**5**, Cu–OH mediated acceleration[17] must be substantial. However, the Cu reagents elicit very little diastereoselectivity in the cleavages of (*RS*)-**5** and (*SS*)-**5**. Kinetic differentiation is minimal, not exceeding a factor of 1.5 (with reagent **6**), even though the 'sense' of the diastereoselectivity does vary with the particular Cu reagent chosen.

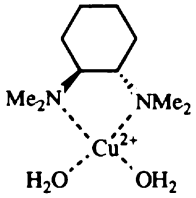
Addition of 5 mM α-CD had little effect on the reaction rates, but β-CD, and especially γ-CD, evoked more interesting responses. As represented in the middle section of Table 1, the addition of β-CD lowered most of the Cu-mediated rate constants. This effect was stronger for (*SS*)-**5** than for (*RS*)-**5**, so that the cleavages, although slower than in the absence of β-CD, became significantly (*RS*) diastereoselective in the presence of β-CD. The largest diastereoselectivity was observed with reagent **8**, where $k_{RS}>k_{SS}$ by ~3.6. Note, however, that ligand identity has little effect on the diastereoselectivity; $Cu(NO_3)_2$ elicits a comparable diastereoselectivity.

The diastereoselectivity developed by β-CD is both reversed and greatly surpassed by γ-CD; see Table 1. Addition of this cyclodextrin strongly retards the Cu-mediated cleavage of (*RS*)-**5**, whereas that of (*SS*)-**5** is either considerably less slowed or even slightly accelerated. The result is a striking diastereoselectivity in which k_{SS} exceeds k_{RS} by factors ranging from 8.5 (with **6**) to 12.7 (with *rac*-**7**). Even $Cu(NO_3)_2$ affords a diastereoselectivity of ~10.

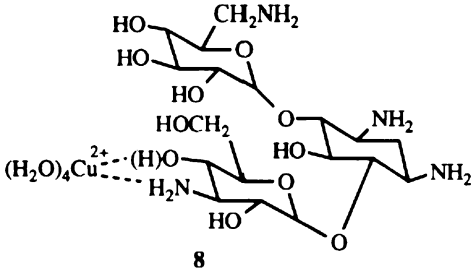
We suggest that the cyclodextrins preferentially bind either (*SS*)-**5** (β-CD) or (*RS*)-**5** (γ-CD). In the latter case, Cu-mediated cleavage of bound (*RS*)-**5** is much less effective than that of free substrate (e.g. k_{RS} is reduced by a factor of 12.8 with *rac*-**7** and γ-CD), whereas the corresponding cleavage of bound (*SS*)-**5** is only marginally reduced (e.g. by a factor of 1.13 with *rac*-7 and γ-CD).[18] The net result is that γ-CD 'protects' (*RS*)-**5** from cleavage more effectively than it shields (*SS*)-**5**, so that diastereoselectivity is strongly amplified.



(1*R*,2*R*)-**6**



(1*R*,2*R*)-**7**



8

Table 1. Rate constants (s^{-1}) for the Cu and CD mediated cleavages of **5**[a]

Cu reagent	Without CD			With 5 mM β-CD			With 5 mM γ-CD		
	$10^3\ k_{RS}$	$10^3\ k_{SS}$	k_{SS}/k_{RS}	$10^3\ k_{RS}$	$10^3\ k_{SS}$	k_{SS}/k_{RS}	$10^3\ k_{RS}$	$10^3\ k_{SS}$	k_{SS}/k_{RS}
6	1.45	2.24	1.5	0.98	0.55	0.56	0.14	1.19	8.5
rac-**7**	21.0	23.5	1.1	8.81	5.24	0.59	1.64	20.8	12.7
(*RR*)-**7**	23.7	19.7	0.83				1.95	21.7	11.1
8	3.21	2.36	0.74	3.62	1.00	0.28	0.35	3.94	11.3
$Cu(NO_3)_2$[b]	2.96	2.88	0.97	2.43	0.76	0.31	0.40	3.94	9.8

[a] Conditions: [**5**]=0.02 mM; [Cu]=1.0 mM, [CD]=5 mM, [HEPES]=20 mM, [KCl]=20 mM, pH 7, 25°C.
[b] Conditions as in a, but at pH 6.0, with the addition of 1 mM CTACl to aid homogeneity.

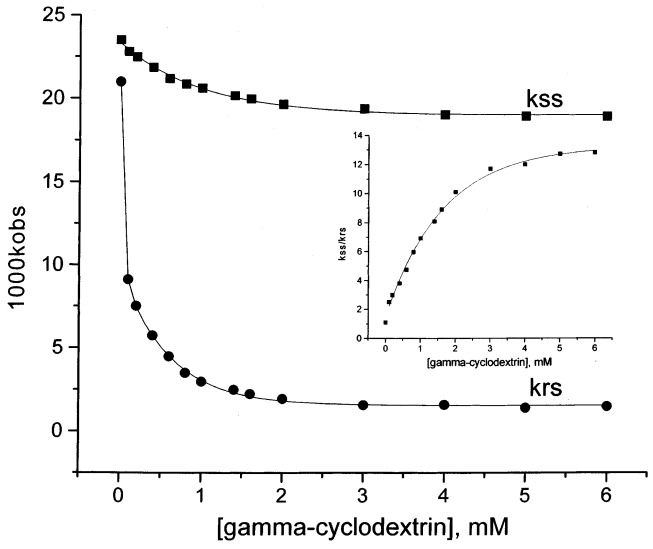


Figure 1. Rate constants (k_{SS} and k_{RS}) for the cleavage of (*SS*)-**5** and (*RS*)-**5** by *rac*-**7** versus the concentration of added γ-CD. *Inset*. Diastereoselectivity (k_{SS}/k_{RS}) versus [γ-CD]. For conditions, see Table 1.

This scenario was studied in detail for the reactions of *rac*-**7** with (*RS*)-**5** and (*SS*)-**5**. Figure 1 portrays the dependence of k_{SS} and k_{RS} on the concentration of γ-CD at pH 7 in the presence of 50-fold excess *rac*-**7**. γ-CD suppresses both reactions, but that of (*RS*)-**5** is clearly slowed more, and more sharply, resulting in a [CD]-dependent kinetic diastereoselectivity favoring the cleavage of (*SS*)-**5**. The inset of Figure 1 depicts the dependence of k_{SS}/k_{RS} on the CD concentration, where the maximum diastereoselectivity is 12.7.

Binding and saturation kinetics are apparent in Figure 1. Non-linear fitting of the data according to Tee et al.[19] afforded values of K_m, the dissociation constants of the γ-CD/**5** complexes: K_m(*SS*)-**5** = 0.96 mM and K_m(*RS*)-**5** = 0.28 mM; the corresponding binding constants, K_b (M^{-1}) are 1047 (*SS*-**5**) and 3548 (*RS*-**5**). Clearly, the *SS*/*RS* diastereoselectivity is not solely due to differential binding of the diastereomers into completely unreactive γ-CD complexes; the ratio of *RS*/*SS* binding constants is only 3.4, whereas with (e.g.) *rac*-**5**, we must account for a diastereoselectivity of 12.7. Moreover, diastereoselectivity in the absence of γ-CD is only 1.1, so that Cu-mediated diastereoselectivity of unbound **5** is unimportant.

Therefore, the observed amplification of the diastereoselectivity arises from differential shielding of the *γ-CD-bound* diastereomers toward Cu-mediated cleavage. (*RS*)-**5** is *both* bound more strongly than (*SS*)-**5** and better protected by the host from the Cu reagent. Bound (*SS*)-**7**, on the other hand, must be so oriented that its Cu-mediated cleavage is little impeded, relative to free (*SS*)-**5**.[20] Note that the diastereoselectivity largely depends on the binding and orientational preferences afforded by the CD host; chiral ligands on the Cu modulate the diastereoselectivity but their identity (and even their presence) are not principal factors.

In conclusion, the substantial observed kinetic diastereoselectivity between phosphonamidothioates (*RS*)-**5** and (*SS*)-**5** is the product of Cu-mediated cleavage coupled with diastereoselective binding and orientational preferences supplied by γ-cyclodextrin.

Acknowledgements

We are grateful to Dr. Barbara A. McKernan for initial experiments, and to the US Army Research Office for financial support.

References

1. Reviews: (a) Morales-Rojas, H.; Moss, R. A. *Chem. Rev.* **2002**, *102*, 2497; (b) Yang, Y.-C. *Acc. Chem. Res.* **1999**, *32*, 109; (c) Séguès, B.; Pérez, E.; Rico-Lattes, I.; Rivière, M.; Lattes, A. *Bull. Soc. Chim. Fr.* **1996**, *113*, 925; (d) Yang, Y.-C.; Baker, J. A.; Ward, J. R. *Chem. Rev.* **1992**, *92*, 1729.
2. (a) Moss, R. A.; Morales-Rojas, H. *Langmuir* **2000**, *16*, 6485; (b) Moss, R. A.; Gong, P. K.; Morales-Rojas, H. *Org. Lett.* **2002**, *4*, 1835; (c) See also Moss, R. A.; Kanamathareddy, S.; Vijayaraghavan, S. *Langmuir* **2001**, *17*, 6108.
3. (a) Breslow, R.; Dong, D. *Chem. Rev.* **1998**, *98*, 1997; (b) D'Souza, V. T.; Lipkowitz, K. B., Eds.; *Chem. Rev.* **1998**, *98*, 8, Number 5, 'Cyclodextrins'; (c) Kirby, A. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 707 (esp. pp. 714–716); (d) Tee, O. S. *Adv. Phys. Org. Chem.* **1994**, *29*, 1; (e) Breslow, R. *Acc. Chem. Res.* **1991**, *24*, 317; (f) *Comprehensive Supramolecular Chemistry*; Zejtli, J.; Osa, T., Eds.; Cyclodextrins; Pergamon, Elsevier: Oxford, 1996; Vol. 3; (g) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer: New York, 1978; (h) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875.
4. (a) van Hooidonk, C.; Breebart-Hansen, J. C. A. E. *Rec. Trav. Chim.* **1970**, *89*, 289; (b) van Hooidonk, C.; Groos, C. C. *Rec. Trav. Chim.* **1970**, *89*, 845.
5. Hoskin, F. C. G.; Steeves, D. M.; Walker, J. E. *Biol. Bull.* **1999**, *197*, 284.
6. (a) Brass, H. J.; Bender, M. L. *J. Am. Chem. Soc.* **1973**, *95*, 5391; (b) Kausar, A. R.; Younas, M.; Sadiq, M. Z.; Wahid, T. *Indian J. Chem.* **1984**, *23B*, 476; (c) Hengge, A. C.; Cleland, W. W. *J. Org. Chem.* **1991**, *56*, 1972.
7. Moss, R. A.; Gong, P. K. *Langmuir* **2000**, *16*, 8551.
8. Barr, L.; Easton, C. J.; Lee, K.; Lincoln, S. F.; Simpson, J. S. *Tetrahedron Lett.* **2002**, *43*, 7797.
9. Vico, R. V.; Buján, E. I.; de Rossi, R. H. *J. Phys. Org. Chem.* **2002**, *15*, 858.
10. Reddy, P. M.; Kovach, I. M. *Tetrahedron Lett.* **2002**, *43*, 4063.
11. We observed for ($Sp S_C$)-**5**: mp 79–80°C (80–81°C),[10] $^{31}P(\delta)$ 78.3 (80.8),[10] and $[\alpha]_D^{25}$ +61.0° (*c* 2, $CHCl_3$) (60.5°).[10] For ($R_P S_C$)-**5**: mp 120–120.5°C (121–122°C),[10] $^{31}P(\delta)$ 79.0 (81.6),[10] and $[\alpha]_D^{25}$ −121.2° (−122°).[10]
12. We thank Dr. Tom Emge of this department for the X-ray structure analysis.
13. (a) Barnes, D. J.; Chapman, R. L.; Vagg, R. S.; Watton, E. C. *J. Chem. Eng. Data* **1978**, *23*, 349; (b) Mulqi, M.;

Stephens, F. S.; Vagg, R. S. *Inorg. Chim. Acta* **1981**, *53*, L91.
14. Catalysts **7** were prepared from *rac*- or (1*R*,2*R*)-1,2-diaminocyclohexanes, which were tetramethylated (5–6-fold excess $H_2C{=}O$, HCOOH, 80°C, 24 h),[15a] and then complexed with $Cu(NO_3)_2$ in ethanol.[15b]
15. (a) Pine, S. H.; Sanchez, B. I. *J. Org. Chem.* **1971**, *36*, 829; (b) Cf. Menger, F. M.; Gan, L. H.; Johnson, E.; Durst, H. D. *J. Am. Chem. Soc.* **1987**, *109*, 2800.
16. Sreedhara, A.; Freed, J. D.; Cowan, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 8814.
17. Scrimin, P.; Ghirlanda, G.; Tecilla, P.; Moss, R. A. *Langmuir*, **1996**, *12*, 6235. See also Ref. 15b.
18. In the absence of a Cu reagent, γ-CD does not cleave the substrates; at pH 7, there is effectively no $CD{-}O^-$ available to attack the phosphonamidothioates.
19. Tee, O. S.; Hussein, S. M. I.; Turner, I. E.; Yazbeck, O. J. *Can. J. Chem.* **2000**, *78*, 436.
20. In several cases (Table 1) γ-CD elicits small increases in k_{SS}, suggesting catalysis, possibly by a CD–Cu complex. Komiyama reports that Ce^{3+} binds to the hydroxyl groups of γ-CD, and that the resulting complexes can cleave phosphoesters: Yoshiro, M.; Miyama, S.; Takarada, T.; Komiyama, M. *J. Incl. Phenom. Mol. Recog. Chem.* **1994**, *17*, 393.