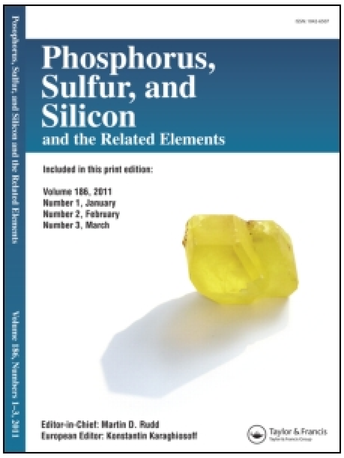

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Charles E. McKenna[a]; Anil Khare[a]; Jing-Yue Ju[a]; Zeng-Min Ll[a]; Greg Duncan[a]; Yung-Chi Cheng[b]; Robert Kilkuskie[c]
[a] Department of Chemistry, University of Southern California, Los Angeles, CA, USA [b] Department of Pharmacology, Yale University, New Haven, CT, USA [c] Cambridge-Biotech, Bethesda, MD, USA

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SYNTHESIS AND HIV-1 REVERSE TRANSCRIPTASE INHIBITION ACTIVITY OF FUNCTIONALIZED PYROPHOSPHATE ANALOGUES

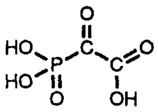
CHARLES E. McKENNA[a], ANIL KHARE[a], JING-YUE JU[a], ZENG-MIN LI[a],
GREG DUNCAN[a], YUNG-CHI CHENG[b] AND ROBERT KILKUSKIE[c]
[a]Department of Chemistry, University of Southern California, Los Angeles, CA
90089-0744 USA; [b]Department of Pharmacology, Yale University, New Haven,
CT 06510-8066 USA; [c]Cambridge-Biotech, Bethesda, MD 20850 USA.

Abstract A new approach, ketone derivatization, for introducing desired functionality into the α-keto pyrophosphate analogues oxophosphonoacetic acid (COPAA) and oxomethanediphosphonic acid (COMDP) is exemplified in the synthesis of several COPAA and COMDP hydrazones with specific functional groups. The preparation of tetraalkyl COMDP esters is also described. Inhibition of HIV-1 reverse transcriptase (isolated enzyme) and of p24 production by HIV-1 (virus-infected H9 cells) by hydrazone derivatives of COPAA and COMDP is briefly discussed.

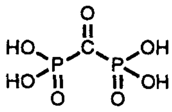
INTRODUCTION

The simple pyrophosphate analogues phosphonoformic acid (PFA), phosphonoacetic acid (PAA) and methanediphosphonic acid (methylenebis[phosphonic acid], MDP) are known to display varying affinities for DNA polymerases and other enzymes. Chemical modification of PFA is limited to alteration of the phosphonate or carboxylate moieties, e.g. by P–O -> P–S replacement[1, 2], whereas PAA and MDP can also be modified at their α-carbon atoms. "Sp^2" analogues of PAA or MDP having a trigonal α-carbon which is one terminus of an unsaturated functionality have been of interest to us. PFA, the only active inhibitor of HIV-1 reverse transcriptase (RT) among the three parent structures, has a trigonal α-carboxylate group, and the α-keto derivatives of PAA (oxophosphonoacetic acid; phosphonoglyoxylic acid, COPAA) and MDP (oxomethanediphosphonic acid,COMDP) are significantly more active than the parents, although less active than PFA[3]. COMDP has also been shown to inhibit mammalian DNA polymerases selectively[4].

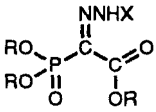
More generally, the synthetically versatile ketone group in COPAA, COMDP and their esters could provide a convenient entry to a range of new α-substituted pyrophosphate analogues. Triethyl COPAA and its bis(dicyclohexylamine) salt ($DCHA_2^+$) were recently prepared by our laboratory[5], and the tetrasodium salt of COMDP has been known for some time[4, 6]. However, esters of COMDP have not been available, nor have possibilities for derivatization of the ketone function in COPAA–COMDP been explored. We report here preliminary results on the synthesis, structure and properties of a new class of pyrophosphate analogues, α-hydrazones of COPAA and COMDP. We also report the related preparation of tetraalkyl COMDP esters. RT inhibition results for some of the compounds are briefly summarized.



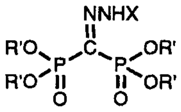
COPAA



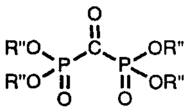
COMDP



1-5, 8-11



6,7,12,16



13-15

R = DCHA, H: X = *E*-Ph (**1**); 4-FPh (**2**); 4-NO_2Ph (**3**); 2,4-$(NO_2)_2$Ph (**4**); 2-MeO-4-NO_2Ph (**5**); Tosyl (**8**); **R' = Na, H**: X = 2,4-$(NO_2)_2$Ph (**6**); $CONH_2$ (**7**); **R = Et**: X = *E*-2-MeO-4-NO_2Ph (**9**); *Z*-2-MeO-4-NO_2Ph (**10**); 4-MeO-2-NO_2Ph (**11**); **R '= Et**: X = 2-MeO-4-NO_2Ph (**12**); 2,4-$(NO_2)_2$Ph (**16**); **R"=** Me (**13**); Et (**14**); *i*Pr (**15**)

RESULTS AND DISCUSSION

Several routes to salts and esters of COPAA and COMDP hydrazones were examined. Because anions of such compounds are usually required for binding to target enzymes, it is particularly convenient to form them directly. Direct synthesis by reaction of COPAA or COMDP with an aryl hydrazine conveniently allows modular variation in the latter structure before assembly of the functionalized phosphonate salt in the final step. We find that the lipophilic ($DCHA_2^+$) salt of COPAA reacts directly with phenyl hydrazine in chloroform at ambient temperature to form the phosphonocarboxylate α–hydrazone salt **1** (62%), which was characterized by NMR, IR, and UV-VIS spectroscopy, and by elemental analysis. An X-ray crystallographic analysis of **1** shows that the phenyl group is *syn* to the carboxyl moiety (*E* geometric isomer). The same method, or a variation of it, was applied to synthesis of a series of aryl hydrazones containing specific functionality in the phenyl moiety: 1) a potential ^{19}F probe (**2**, 75%); 2) auxochromes (**3**, 63%; **4**, 78%); and 3) 4-nitro-2-methoxy substituents, which create a photochemically reactive aryl hydrazone (**5**, 79%) that strongly absorbs light in the visible region (λ_{max} = 410 nm, ε = 2.76×10^4 in 98% aq. MeOH v/v). The strong peak at 410 nm in a 0.002% solution of **5** gradually diminished on exposure to sunlight over several hours and vanished when the solution was irradiated in a Rayonet photoreactor (GE G8 15 mercury lamps). Compound **6** was formed by reaction of the tetrasodium salt of COMDP with the corresponding aryl hydrazine in aqueous alcohol-HCll. A similar method when used with semicarbazide gave an adduct presumed (NMR) to be **7**, but with phenyl hydrazine failed to give an isolable COMDP hydrazone corresponding to **1**. Instead, a ^{31}P NMR peak at $\delta = -15$ ppm was observed, which may have originated from hydrolytic breakdown of the desired product. The influence of the hydrazone **X** group (**Scheme**) and phosphonate structure on the relative stability of COPAA andCOMDP hydrazones in aqueous solution is under further investigation.

We also examined complementary preparations of trialkyl COPAA and tetraalkyl COMDP hydrazones by : 1) reaction of aryl diazonium partners with PAA or MDP esters, or 2) direct aryl hydrazine condensation with the α-keto esters. To demonstrate the first method, the *o,p*-isomeric methoxynitrophenyl hydrazones of COPAA, (**9**, 62%; **11**, 60%) were prepared by diazotizing the proper methoxynitroaniline at 0° in 4% HCl, adding a concentrated solution of triethyl PAA in H_2O, and adjustment of the pH to 9–10 with 10 M NaOH. The products were characterized by NMR and UV-VIS spectroscopy, and by elemental analysis. The same method applied to tetraethyl MDP with 2-methoxy-4-nitroaniline gave a product characterized as the corresponding hydrazone (**12**) by ^{31}P, ^{1}H and ^{13}C NMR.

X-ray crystallographic analysis of **9** revealed that the phenyl group is *syn* to the carboxylate moiety, as was the case for the phenylhydrazone salt **1**. Both compounds were stable in solution at ambient temperature (**1**: sl. alk. H_2O; **9**: $CDCl_3$). However, when an acetone solution of **9** was heated in a sealed tube at 78 °C for several hours, a new peak appeared in the ^{31}P NMR spectrum of the solution. The ^{1}H and ^{13}C NMR spectra revealed that most of the original signals were distinguishably doubled by new peaks at slightly different chemical shifts, consistent with partial conversion to the *Z* isomer.

Application of the classical route (direct reaction with a hydrazine) to hydrazones of COPAA and COMDP esters required access to the latter compounds. Oxygen transfer from propylene oxide, which affords triethyl COPAA from triethyl α-diazoPAA[5], proved ineffective with α-diazoMDP esters, which were unreactive with the Rh^{II} catalyst used. Attempted oxidation of the methylene group in tetraalkyl MDP by oxidants such as N_2O_4, which convert diethyl malonate to α-oxomalonate, was also unsuccessful[7]. However, halo-formylation[8] of tetramethyl, -ethyl or -isopropyl MDP esters in HCO_2H by treatment with a slight excess of *t*BuOCl, followed by heating at reduced pressure for 2 h, produces the desired ketones (**13-15**) in moderate yields, accompanied by a small amount of α,α-dichlorinated (^{31}P, ^{13}C NMR) side product. The COMDP esters are brilliant yellow liquids (visible λ_{max} = 412–3 nm), which were characterized by ^{31}P, ^{1}H and ^{13}C NMR, MS, and by IR ($\nu_{C=O} \approx$ 1645 cm $^{-1}$, neat). They immediately react with H_2O quantitatively to give *gem*-diols (≈ 20 ppm downfield shift of ^{31}P NMR resonance). In a typical hydrazone derivatization, **13** was stirred with a small excess of 2,4-dinitrophenylhydrazine in $CHCl_3$ at ambient temperature for 1 h, giving **15** (95%), characterized by NMR, UV-visible spectroscopy, and elemental analysis.

Evaluation of a selected group (**2-4**, **6-8**) of hydrazone derivatives (c = 15 µM) with isolated HIV-1 reverse transcriptase (poly(rC)p(dG)) gave inhibitions of 15%—45%, comparable to or somewhat less than the α-keto parents (COPAA, COMDP) but greater than that of PAA or MDP themselves, which are inactive at this concentration. When screened for inhibition of p24 production by virus-infected H9 cells, the MDP semicarbazone **7** was slightly inhibitory at 100 µM and the other derivatives were inhibitory only at toxic concentrations. It should be noted that the enzyme assays were conducted over intervals of a few minutes, whereas the p24 assay is done over several days. While these results indicate that the particular compounds tested here have no therapeutic potential for HIV as such, they also demonstrate that a C=N-R function, where R is a large group, can replace the α-keto group in COPAA or COMDP without necessarily abolishing polymerase (RT) affinity. Moreover, we note a recent report that the phenyl hydrazone **1** inhibits Na^+ phosphate cotransport in renal membrane and thus may provide a new approach for introducing desirable functionality into analogues targeting this specific orthophosphate binding site[9] .

ACKNOWLEDGMENTS

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