Synthesis and Evaluation of Peptidyl Vicinal Tricarbonyl Monohydrates as Inhibitors of Hydrolytic Enzymes

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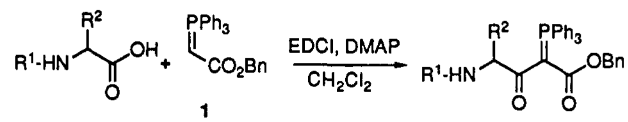
Summary: Peptidyl vicinal tricarbonyls, prepared from *N*-protected di- and tripeptides by reaction of the carboxylic acid residues with ylides followed by oxidation, have been shown to be potent inhibitors of serine proteases.

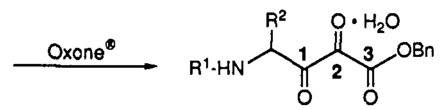
There has been considerable recent interest in small peptide substrate analogues which incorporate a strongly electron-deficient group at the site of the scissile amide unit and thereby act as potent inhibitors of hydrolytic enzymes such as α-chymotrypsin, human neutrophil elastase (HNE), and porcine pancreatic elastase (PPE).[1] The active carbonyls in groupings such as trifluoromethyl ketones (TFMK's),[2] α-diketones,[3] and α-keto esters[4] appear to be excellent acceptors for nucleophilic residues such as the serine hydroxyl or the thiol group of a cysteine unit in the enzyme molecule. Inhibition has been associated with the formation of a tetrahedral intermediate, which, in the case of serine, would be a hemiketal.[1]

Among functional groups in which the electrophilic reactivity of the carbonyl group is greatly enhanced, the vicinal tricarbonyl unit stands out as one of the most powerful acceptors.[5] The central (C_2) carbonyl group in this system is destabilized by dipolar effects in the ground state and forms a stable monohydrate which, in solution, is in equilibrium with the parent tricarbonyl group. The C_1 carbonyl, adjacent to the strongly electron-attracting C_2–C_3 α-dicarbonyl array, is also highly activated and readily forms hemiacetals in suitable environments as in FK-506[6] and rapamycin.[7] Reactions of these species with donor molecules take place rapidly to form tetrahedral intermediates. In the setting of a small peptide aggregate, which is suitable for binding to the enzyme, one would expect such tricarbonyl derivatives to behave as potent enzyme inhibitors analogous to the corresponding TFMK's.[2]

Scheme I



2a; R^1 = Cbz, R^2 = CH_2Ph (79%)
2b; R^1 = Boc, R^2 = H (62%)
2c; R^1 = Cbz, R^2 = CH_3 (77%)



3a; R^1 = Cbz, R^2 = CH_2Ph (66%)
3b; R^1 = Boc, R^2 = H (64%)
3c; R^1 = Cbz, R^2 = CH_3 (70%)

In our recent studies on the chemistry of vicinal tricarbonyl compounds, we have developed a mild, efficient reaction sequence for forming the 1,2,3-tricarbonyl aggregate from carboxylic acid precursors.[8] This method, outlined below, has now been applied to the formation of tricarbonyl esters containing dipeptide and tripeptide residues.

Scheme I illustrates the general procedure by which the carboxylic acid (1 equiv)[9] is coupled with an ylide 1 (1 equiv) in the presence of EDCI (1 equiv)[10] and DMAP (catalytic) to form the keto phosphorane 2[11] which can then be subsequently oxidized (1.5 equiv of Oxone in THF/H_2O)[12] to the hydrated tricarbonyl derivative 3. Using peptidyl carboxylic acids as starting materials in this way,

(1) (a) Trainor, D. A. *Trends Pharmacol. Sci.* **1987**, *8*, 303–307. (b) Stein, M. M.; Wildonger, R. A.; Trainor, D. A.; Edwards, P. D.; Yee, Y. K.; Lewis, J. J.; Zottola, M. A.; Williams, J. C.; Strimpler, A. M. In *Peptides, Chemistry, Structure, and Biology* (Proceedings of the Eleventh American Peptide Symposium); River, J. E., Marshall, G. R., Eds.; ESCOM: Leiden, 1990; pp 369–370. (c) Edwards, P. D.; Meyer, E. F., Jr.; Vijayalakshmi, J.; Tuthill, P. A.; Andisik, D. A.; Gomes, B.; Strimpler, A. *J. Am. Chem. Soc.* **1992**, *114*, 1854–1863. (d) Stein, R. L.; Strimpler, A. M.; Edwards, P. D.; Lewis, J. J.; Mauger, R. C.; Schwartz, J. A.; Stein, M. M.; Trainor, D. A.; Wildonger, R. A.; Zottola, M. A. *Biochemistry* **1987**, *26*, 2682–2689.

(2) (a) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813–1817. (b) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*, 3760–3767.

(3) (a) Angelastro, M. R.; Mehdi, S.; Burkhart, J. P.; Peet, N. P.; Bey, P. *J. Med. Chem.* **1990**, *33*, 11–13. (b) Mehdi, S.; Angelastro, M. R.; Burkhart, J. P.; Koehl, J. R.; Peet, N. P.; Bey, P. *Biochem. Biophys. Res. Commun.* **1990**, *166*, 595–600.

(4) (a) Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. J. *J. Med. Chem.* **1990**, *33*, 394–407. (b) Buckhart, J. P.; Peet, N. P.; Bey, P. *Tetrahedron Lett.* **1990**, *31*, 1385–1388.

(5) (a) Wasserman, H. H.; Han, W. T. *J. Am. Chem. Soc.* **1985**, *105*, 1444–1446. (b) Wasserman, H. H.; Han, W. T. *Tetrahedron Lett.* **1984**, *25*, 3743–3746. (c) Wasserman, H. H.; Han, W. T. *Tetrahedron Lett.* **1984**, *25*, 3747–3750. (d) Wasserman, H. H.; Amici, R.; Frechette, R.; van Duzer, J. H. *Tetrahedron Lett.* **1989**, *30*, 869–872. (e) Wasserman, H. H.; Kuo, G-H. *Tetrahedron Lett.* **1989**, *30*, 873–876. (f) Wasserman, H. H.; Kelly, T. A. *Tetrahedron Lett.* **1989**, *30*, 7117–7120. (g) Wasserman, H. H.; Rotello, V. M.; Williams, D. R.; Benbow, J. W. *J. Org. Chem.* **1989**, *54*, 2785–2786. (h) Wasserman, H. H.; Ennis, D. S.; Vu, C. B. *Tetrahedron Lett.* **1991**, *32*, 6039–6042.

(6) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031.

(7) (a) Sehgal, S. N.; Baker, H.; Vézina, C. *J. Antibiot.* **1975**, *28*, 727. (b) Swindells, D. C. N.; White, P. S.; Findlay, J. A. *Can. J. Chem.* **1978**, *56*, 2491.

(8) Wasserman, H. H.; Ennis, D. S.; Rotello, V. M.; Blum, C. A. *Tetrahedron Lett.* **1992**, *33*, 6003–6006.

(9) *N*-Protected α-amino acids were purchased from the Sigma Chemical Co. as either the enantiomerically enriched material or as a racemic mixture of enantiomers.

(10) Preliminary results indicate that the BOP ((benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) reagent provides an alternative method for generating the keto ylides in comparable yields. BOP reagent: (a) Coste, J.; Frerot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, *32*, 1967–1970. (b) Castro, B.; Evin, G.; Selve, C.; Seyer, R. *Synthesis* **1977**, 413. (c) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219–1222.

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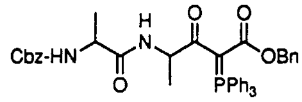
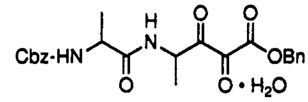
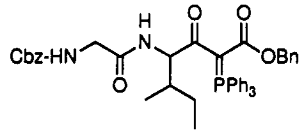
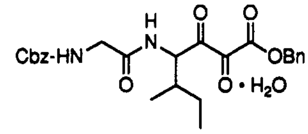
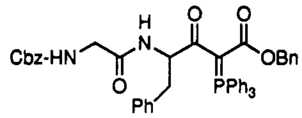
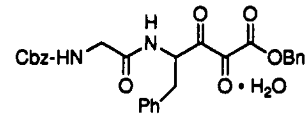
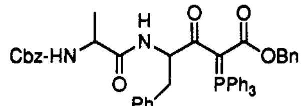
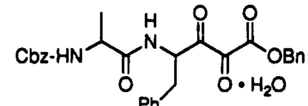
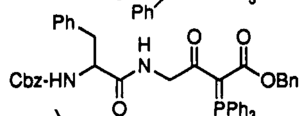
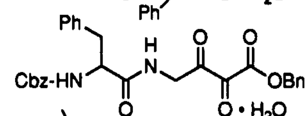
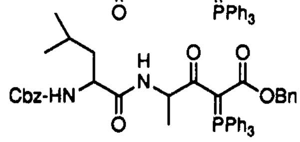
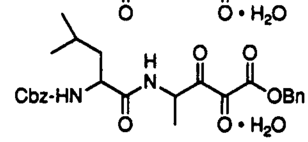
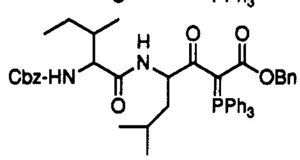
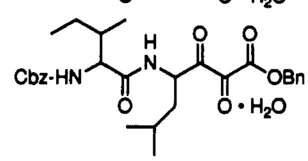
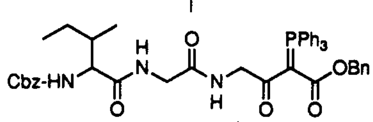
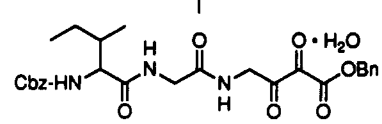
Table I. Formation of Peptidyl Tricarbonyls via Intermediate Peptidyl Keto Ylides

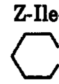
entry	peptide	keto ylide		yield (%)	tricarbonyl		yield (%)
1	Cbz-HN-Ala-Ala		4	(50)		12	(79)
2	Cbz-HN-Gly-Ile		5	(54)		13	(67)
3	Cbz-HN-Gly-Phe		6	(62)		14	(67)
4	Cbz-HN-Ala-Phe		7	(69)		15	(51)
5	Cbz-HN-Phe-Gly		8	(52)		16	(58)
6	Cbz-HN-Leu-Ala		9	(49)		17	(52)
7	Cbz-HN-Ile-Leu		10	(58)		18	(68)
8	Cbz-HN-Ile-Gly-Gly		11	(41)		19	(15)

we have now prepared the tricarbonyl monohydrates 12–19 (Table I).

The tricarbonyl derivatives were generally isolated as pale green oils, although several samples have crystallized *in vacuo*. They may be stored at 0 °C for prolonged periods (>6 months) without observable decomposition. Spectroscopic studies (NMR, IR) confirmed that they exist in the hydrated form. In the IR, peaks at 3600–3300 cm^{-1} are consistent with hydroxyl stretching of the hydrates. In the case of 14 (entry 3, Table I), ^{13}C NMR shows a signal at δ 93 ppm, in accord with the known ^{13}C resonance of related 1,1-diols.[13] For each of the products, high-resolution mass spectrometry (CI) gave parent molecular ions in exact agreement with the tricarbonyl forms.[14]

All of the tricarbonyl monohydrates listed in Table I have now been tested for activity as enzyme inhibitors in standard assay procedures[1c] with the collaboration of Zeneca Pharmaceuticals. The results of these studies are summarized in Table II. The compounds were tested against two closely related serine proteases, HNE and PPE. Moderately high potency was achieved against HNE, and some degree of selectivity between the elastases was demonstrated. Although the same peptides with other electrophilic termini (e.g., TFMK's[2], α-diketones[3], α-keto esters[4]) were not available for comparison, the overall potency of the tricarbonyl compounds appears to be

Table II. Peptidyl Tricarbonyls as Inhibitors of HNE, PPE, and α-Chymotrypsin

entry		peptide tricarbonyls	K_i HNE (μM)	K_i PPE (μM)	K_i α-Chy (μM)
1	12	Z-Ala-Ala-$(CO)_3$-OBn·H_2O	0.80	NI	210
2	13	Z-Gly-Ile-$(CO)_3$-OBn-·H_2O	5.1	NI	1.6
3	14	Z-Gly-Phe-$(CO)_3$-OBn·H_2O	1.6	NI	0.11
4	15	Z-Ala-Phe-$(CO)_3$-OBn·H_2O	1.4	30	0.3
5	16	Z-Phe-Gly-$(CO)_3$-OBn·H_2O	2.95	47	4.9
6	17	Z-Leu-Ala-$(CO)_3$-OBn·H_2O	400	110	6.2
7	18	Z-Ile-Leu-$(CO)_3$-OBn·H_2O	959	NI	
8	19	Z-Ile-Gly-Gly-$(CO)_3$-OBn·H_2O	0.69	NI	
9	22	 N—$(CO)_3$—CH_2—NH—Fmoc·H_2O	240	NI	

(11) All of the ylides were fully characterized with elemental analyses, ^1H NMR, IR, and melting points. No epimerization of enantiomerically enriched Cbz-NH-*L*-Ala, $[\alpha]^{23} = -14.2°$ (c = 2, CH_3CO_2H), was observed during the coupling procedure. Product 2c showed a specific rotation, $[\alpha]^{21} = +12.45°$ (c = 2, $CDCl_3$), and none of the opposite enantiomer was detected by ^1H NMR experiments employing the lanthanide shift reagent, $Eu(fod)_3$.

(12) Wasserman, H. H.; Vu, C. B. *Tetrahedron Lett.* **1990**, *31*, 5205–5208.

(13) Jones, T. K.; Reamer, R. A.; Desmond, R.; Mills, S. G. *J. Am. Chem. Soc.* **1990**, *112*, 2998–3017.

(14) Rapid loss of water from the hydrates would be expected under the conditions of chemical ionization mass spectrometry.

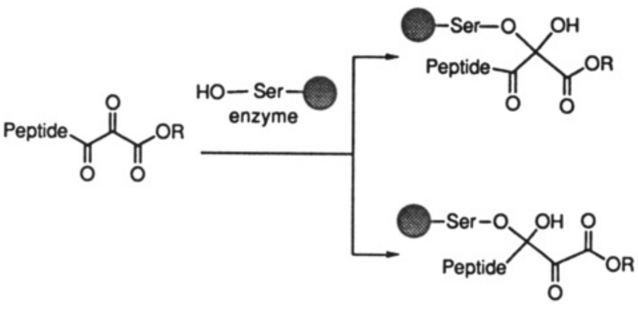


Figure 1. Covalent bond-forming reaction between the peptidyl tricarbonyl substrate and the hydroxyl group of the active site serine leading to formation of the tetrahedral adduct.

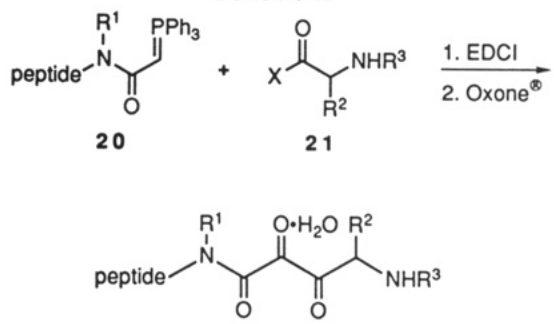
comparable to that observed with these types of inhibitors.[15] It is interesting to note that tricarbonyls **14** and **15** (Table II, entries 3 and 4) which possess a phenylalanine residue in the P_1 position[16] adjacent to the tricarbonyl array showed potent inhibitory activity toward α-chymotrypsin, an enzyme known to demand a hydrophobic aromatic residue on the carbonyl side of the scissile amide bond.

Serine proteases HNE and PPE differ from α-chymotrypsin in that the binding area is far more extended, and thus, they are more specific for longer chain peptides.[1] Tripeptidyl tricarbonyl (**19**) (Table II, entry 8) was the most potent HNE inhibitor of our synthetic peptido tricarbonyls, in accord with the expected effect of the extended binding site.

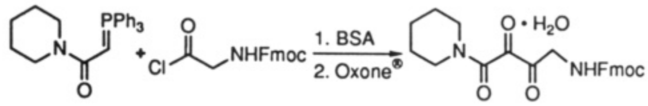
By analogy with peptidyl aldehydes, α-keto esters, and TFMK's, we presume that the mechanism for inhibition of serine proteases by the peptidyl tricarbonyls involves formation of a stable hemiketal adduct resulting from attack by the active site serine, either at the central (C_2) carbonyl or at the C_1 carbonyl of the tricarbonyl moiety (Figure 1). In the latter circumstance, the reacting carbonyl might be more favorably positioned relative to the scissile amide bond of the corresponding peptide substrate.

Current work in our laboratories is now directed toward the structural modification of the most active dipeptidyl tricarbonyls in an attempt to improve upon the inhibitory properties. Along the lines of our earlier studies related to FK506[5g] and bicyclomycin,[17] we are also exploring the

Scheme II



Scheme III



coupling of amido ylides (**20**) with activated carboxylates (**21**) as a route to the generation of a tricarbonyl residue introduced at the *N*-terminus of a peptide chain (Scheme II).

In our preliminary work in this direction, tricarbonyl **22** has been prepared by BSA-mediated coupling[8,18] of amido phosphoranylidene **23**[5g] and *N*-Fmoc-protected glycyl acid chloride,[19] followed by standard oxidative cleavage (Scheme III). Product **22** was assayed against several serine proteases and exhibited moderate inhibition of HNE (Table II, entry 9).

In conclusion, we have shown that peptido carboxylic acids are readily converted into peptidyl tricarbonyls in two mild steps: (1) EDCI-promoted coupling with ylide **1** and (2) oxidation.[20] Of the tricarbonyl derivatives thus formed, several products exhibit potent inhibition of hydrolytic enzymes.

Acknowledgment. This research was supported by NIH Grant GM 07874. P.L.P. thanks NSERC (Canada) for a postdoctoral fellowship.

Supplementary Material Available: Experimental details and spectroscopic data for all new compounds (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(15) Examples of K_i values for related serine protease inhibitors include the following: (1) Ac-Ala-Phe-CF_3, K_i (α-chymotrypsin) = 11.0 μM (Brady, K.; Abeles, R. H. *Biochemistry* **1990**, *29*, 7608–7617); (2) Ac-Gly-Phe-CF_3, K_i (α-chymotrypsin) = 18 μM (*Ibid.*); (3) Cbz-Pro-Val-CF_3, K_i (HNE) = 1.8 μM (ref 1d); (4) Cbz-Val-Phe-CO_2Me, K_i (α-chymotrypsin) = 0.06 μM (ref 3a); (5) Boc-D-Phe-Pro-Val-CF_3, K_i (HNE) = 0.16 μM, K_i (PPE) = 1.8 μM (ref 4a).

(16) The terminology used to describe residues was originally proposed by Schecter and Berger (1967). The amino acid residues of substrates (or inhibitors) are designated P_1, P_2, etc. numbering from the carbonyl of the scissile amide bond in the direction of the amino terminal.

(17) Wasserman, H. H.; Rotello, V. M.; Krause, G. B. *Tetrahedron Lett.* **1992**, *33*, 5419–5422.

(18) Fmoc-glycine, activated by EDCI or BOP, failed to couple with amido phosphoranylidene **20**.

(19) Carpino, L. A.; Cohen, B. J.; Stephens, Jr., K. E.; Sadat-Aalaee, S. Y.; Tien, J-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732–3734 and references cited therein.

(20) In general, oxidative cleavage can be accomplished more rapidly with ozone; however, Oxone provides a milder and more selective reagent.