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A Novel Phosphotyrosine Mimetic 4′-Carboxymethyloxy-3′-phosphonophenylalanine (Cpp): Exploitation in the Design of Nonpeptide Inhibitors of pp60^{Src} SH2 Domain†

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Abstract—The novel phosphotyrosine (pTyr) mimetic 4′-carboxymethyloxy-3′-phosphonophenylalanine (Cpp) has been designed and incorporated into a series of nonpeptide inhibitors of the SH2 domain of pp60$^{c\text{-}Src}$ (Src) tyrosine kinase. A 2.2 Å X-ray crystal structure of **1a** bound to a mutant form of Lck SH2 domain provides insight regarding the structure–activity relationships and supports the design concept of this new pTyr mimetic. © 2001 Elsevier Science Ltd. All rights reserved.

The ubiquitous nature of protein kinases and their significant roles in signal transduction have prompted the examination of these proteins as therapeutic targets. In particular, inhibitors of pp60$^{c\text{-}Src}$ (Src) tyrosine kinase have been identified as potential therapeutics for osteoporosis[1–3] and cancer.[4–6] The kinase possesses a noncatalytic domain referred to as the Src homology domain-2 (SH2) that is characterized by a sequence-specific phosphotyrosine (pTyr) binding region. The SH2 domain is known to be essential for propagating intracellular signals through pTyr recognition leading to an assemblage of multiprotein complexes.[7] Src SH2 inhibitors have been developed to block such signal transduction pathways.[8–10]

The X-ray crystal structure of the pTyr containing pentapeptide Ac-pTyr-Glu-Glu-Ile-Glu complexed with Src SH2 has been solved.[8] Such data have shown a distinct pTyr binding region, hydrophobic pockets and potential electrostatic and hydrogen bonding contacts and have provided a starting point for the design of nonpeptidic ligands for SH2 domains. Recently, Lunney et al.[9] and Sawyer[10] have described a modified benzamide as an effective nonpeptidic template to replace the Glu-Glu-Ile substructure of the above phosphopeptide. Nevertheless, the pharmacological application of pTyr containing analogues is limited by the metabolic liabilities of the phosphate group. Extensive efforts have resulted in a large number of nonhydrolyzable pTyr analogues.[11–17] Our recent efforts to obtain novel nonhydrolyzable pTyr analogues have shown that the incorporation of a phosphonate in the 3′-position of pTyr yielded high affinity ligands for Src SH2.[18] In an effort to further explore novel 3′,4′-difunctionalized pTyr mimics, the design and synthesis of 4′-carboxymethyloxy-3′-phosphonophenylalanine (Cpp) modified series of nonpeptide inhibitors of the Src SH2 domain was undertaken.

The novel pTyr mimetic was assembled by coupling a functionalized aryl iodide to an iodo-zinc alanine derivative (Scheme 1). To prepare the aryl iodide, diethyl(2-hydroxyphenyl)phosphonate (**2**) was obtained by treating diethyl(2-iodophenyl)phosphate with butyllithium.[19] Regioselective iodination was successfully accomplished using chloramine-T and sodium iodide.[20] Protection of the phenol as a benzyl ether gave **3**, which was then used for the coupling to Boc-protected iodo-zinc alanine derivative.[21] The benzyl group of **4** was removed by hydrogenation and saponification gave the Boc-protected

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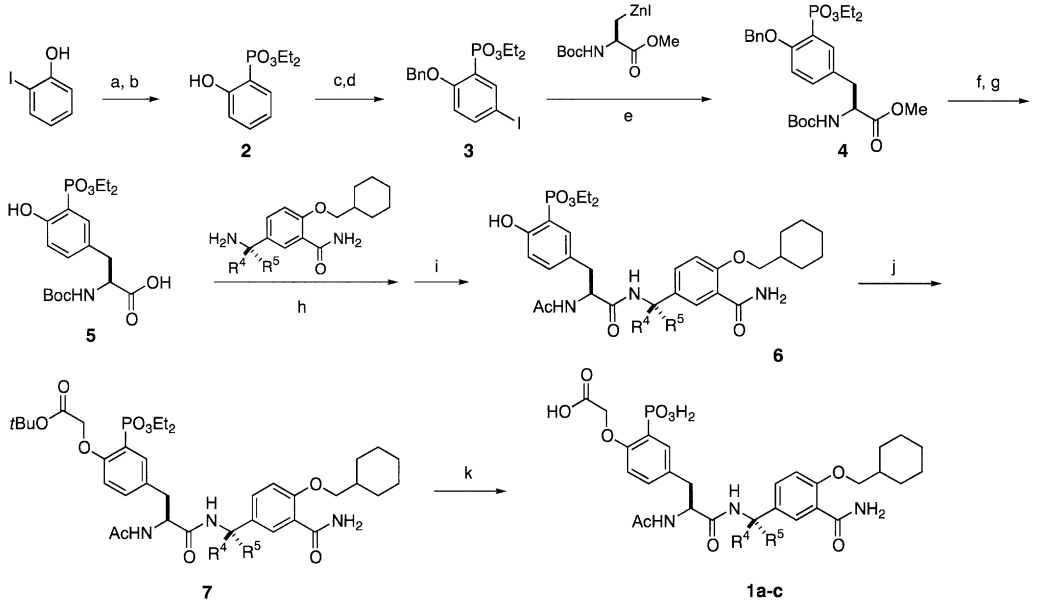
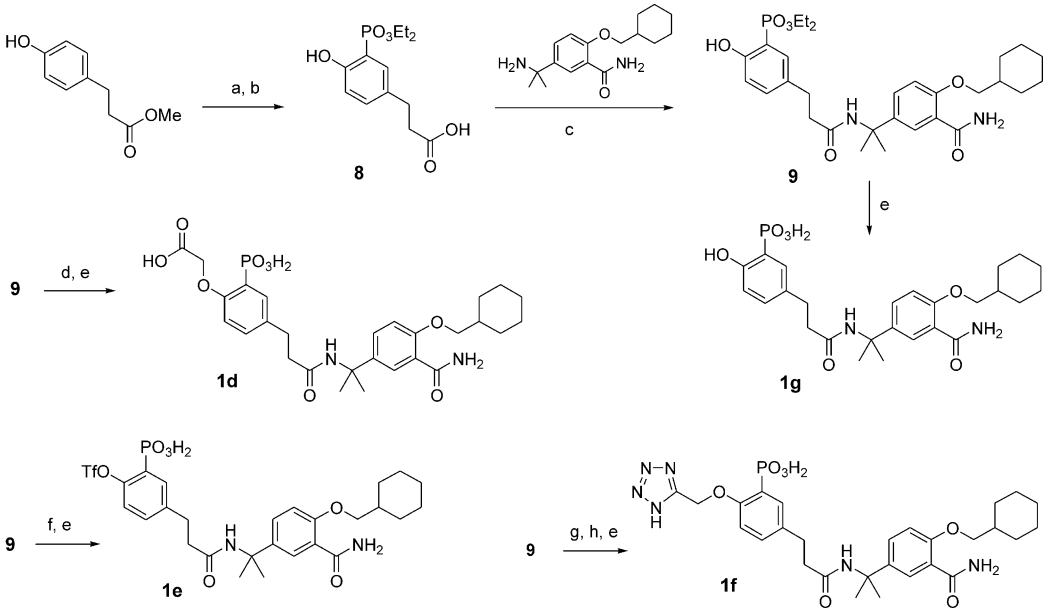
3′-diethylphosphonotyrosine derivative (**5**). The stereochemical integrity of **5** was confirmed by coupling to optically pure (*R*)- and (*S*)-1-phenethylamine using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxy-7-azabenzotriazole (HOAt). A comparison of the diastereomers by NMR indicated that this novel pTyr mimetic is obtained in high enantiomeric purity (>97%). The carboxylic acid **5** was then coupled to the benzamide templates[9,10,22] using EDC and HOAt. Exchange of the Boc group for an acetyl, *O*-alkylation and deprotection using iodotrimethylsilane (TMSI) gave the desired final products **1a–c** after purification by reversed-phase HPLC (RP-HPLC) and lyophilization. A monoethylphosphonate analogue **1h** was obtained by quenching the TMSI-mediated deprotection prior to full deprotection of the phosphonate.

A *des*-acetylamino series was synthesized to explore the effect of removing the acetylamino moiety thereby eliminating a chiral center (Scheme 2). Additionally, modifications to the carboxymethyl group were examined within this series. Methyl-3-(4-hydroxyphenyl)propionate was phosphorylated and an *ortho*-transfer of phosphate was carried out in a manner similar to that described above except without lithium halogen exchange to give

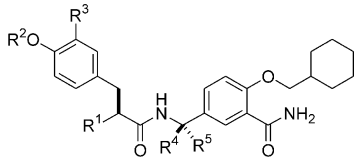


Scheme 1. Synthesis and incorporation of the Cpp residue: (a) Et_2O_3PCl, K_2CO_3, MeCN, 95%; (b) BuLi, THF, −78 °C, 93%; (c) chloramine-T, NaI, DMF, 73%; (d) BnBr, Cs_2CO_3, MeCN, 91%; (e) $(PhCN)_2PdCl_2$ (*o*-tol)$_3$P, DMA, THF, 63%; (f) H_2, Pd/C; (g) LiOH; (h) EDC, HOAt, NMM, 75%; (i) TFA, DCM then Ac_2O, NMM, 83%; (j) $BrCH_2COOt$-Bu, Cs_2CO_3, MeCN, 89%; (k) TMSI, MeCN.



Scheme 2. Synthesis of the *des*-acetylamino series: (a) Et_2O_3PCl, K_2CO_3, MeCN, 96%; (b) (i) LDA, THF, −78 °C, 65%; (ii) LiOH, MeOH, THF, 90%; (c) EDC, HOAt, NMM, 82%; (d) $BrCH_2CO_2t$-Bu, Cs_2CO_3, 89%; (e) TMSI, MeCN; (f) Tf_2NPh, TEA, 88%; (g) $BrCH_2CN$, Cs_2CO_3, 95%; (h) NaN_3, NH_4Cl.

Table 1. SH2 binding affinities (IC_{50}, μM) of **1a**–**k** in the fluorescence polarization[23] assay



Compound	R^1	R^2	R^3	R^4	R^5	SH2 binding (IC_{50})		
						Src	Yes	Zap70
1a	Ac-HN–	$HO_2C–CH_2$–	H_2O_3P–	H–	H_3C–	3.6	110	> 500
1b	Ac-HN–	$HO_2C–CH_2$–	H_2O_3P–	H_3C–	H_3C–	3.2	94.5	> 500
1c	Ac-HN–	$HO_2C–CH_2$–	H_2O_3P–	H–	H–	378	> 500	> 500
1d	H–	$HO_2C–CH_2$–	H_2O_3P–	H_3C–	H_3C–	35.4	303	> 500
1e	H–	F_3CO_2S–	H_2O_3P–	H_3C–	H_3C–	> 500	> 500	> 500
1f	H–	tetrazole–H_2C–	H_2O_3P–	H_3C–	H_3C–	> 500	> 500	> 500
1g	H–	H–	H_2O_3P–	H_3C–	H_3C–	> 500	> 500	> 500
1h	Ac-HN–	$HO_2C–CH_2$–	$EtHO_3P$–	H–	H_3C–	7.3	156	> 500
1i[a]	Ac-HN–	$HO_2C–CH_2$–	H_2O_3P–	—	—	> 500	> 500	> 500
1j	Ac-HN–	$HO_2C–CH_2$–	H–	H–	H_3C–	370	> 500	—
1k	Ac-HN–	H_2O_3P–	H–	H–	H_3C–	2.7	2.6	441

Binding affinity assessed by IC_{50} (μM) using the fluorescent analogue of Ac-pTyr-Glu-Glu-Ile-Glu as described in Lynch et al.[23]
[a]Control compound (Ac-Cpp-benzylamide).

8.[19] Coupling to the *gem*-dimethyl benzamide template followed by *O*-derivatization and deprotection provided the *des*-acetylamino analogues **1d**–**g** after RP-HPLC purification and lyophilization.

The binding affinities of **1a**–**k** to the Src SH2 domain (Table 1) were determined using a fluorescence polarization (FP) binding assay.[23] The binding affinity of **1a** is comparable to the corresponding pTyr-containing analogue **1k**. Noteworthy, **1a** is noticeably more selective than **1k** for the Src SH2 versus the Yes SH2. Additionally, the dramatic increase in binding affinity of **1a** relative to **1j** highlights the importance of the 3′-phosphonate. Similar binding affinity and Src SH2 selectivity were observed for **1b**, which possesses the geminal dimethyl benzamide template. The importance of the methyl groups of the benzamide template is highlighted by the diminished binding affinity of **1c** relative to **1a** and **1b**. It is also interesting to note that the monoethyl phosphonate analogue **1h** displays a decrease in binding affinity of only 2-fold relative to **1a**. The 10-fold loss in binding affinity of **1d** indicates the importance of the acetylamino group. Replacement of the carboxymethyloxy group with a triflate, tetrazolemethylether, or hydrogen (**1e**–**g**, respectively) abolishes activity indicating the importance of the carboxylic acid functionality.

The rabbit osteoclast pitting assay[24] was performed to assess the ability of the compound to penetrate the cells and inhibit rabbit osteoclast-mediated resorption of dentine. Compound **1a** was observed to weakly inhibit the resorption of dentine slices by rabbit osteoclasts (Table 2). An increase in the inhibitory properties was observed for **1h** and it is believed that this is due to enhanced cellular permeability of the compound.

Table 2. Cellular antiresportive activity in the rabbit osteoclast pitting assay[24]

Compound	% Inhibition[a]
1a	+
1h	+ + +
1k	+ +

[a]Where '+' = 0–35% inhibition at 100 μM, '+ +' = 36–70% inhibition at 100 μM and '+ + +' = 71–100% inhibition at 100 μM.

Compound **1a** was co-crystallized with a mutant Lck SH2 (S164C substituted Lck119-225) that possesses matched binding pocket residues with that of Src SH2 (Fig. 1).[25] The 4′-carboxymethylether moiety of the Cpp is observed to occupy the phosphate binding pocket. Relative to **1k**, the phenyl ring of the Cpp residue of **1a** is shifted out of the aromatic binding pocket. This may result from the increase in the number of atoms between the aromatic ring and the carboxylic acid relative to the phosphate of pTyr. This positioning of the aromatic ring allows for the extension of the Lys βD6 side chain (Fig. 2a and b) and places the side-chain ε-amino group

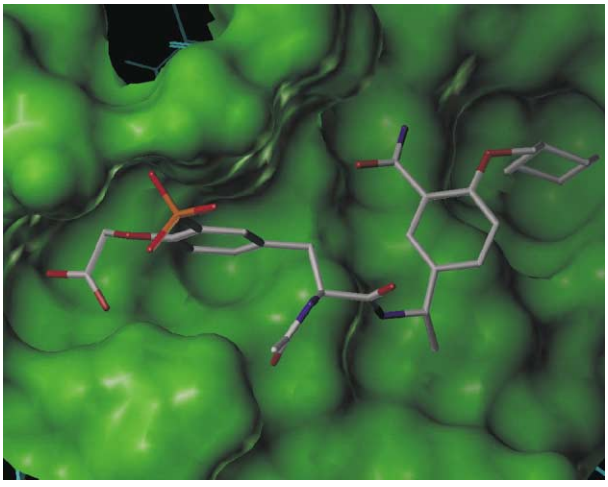


Figure 1. X-ray crystallographic structure of **1a** bound to a mutant of Lck (119-225) SH2 (Lck119-225 S164C) (2.2 Å).[25]

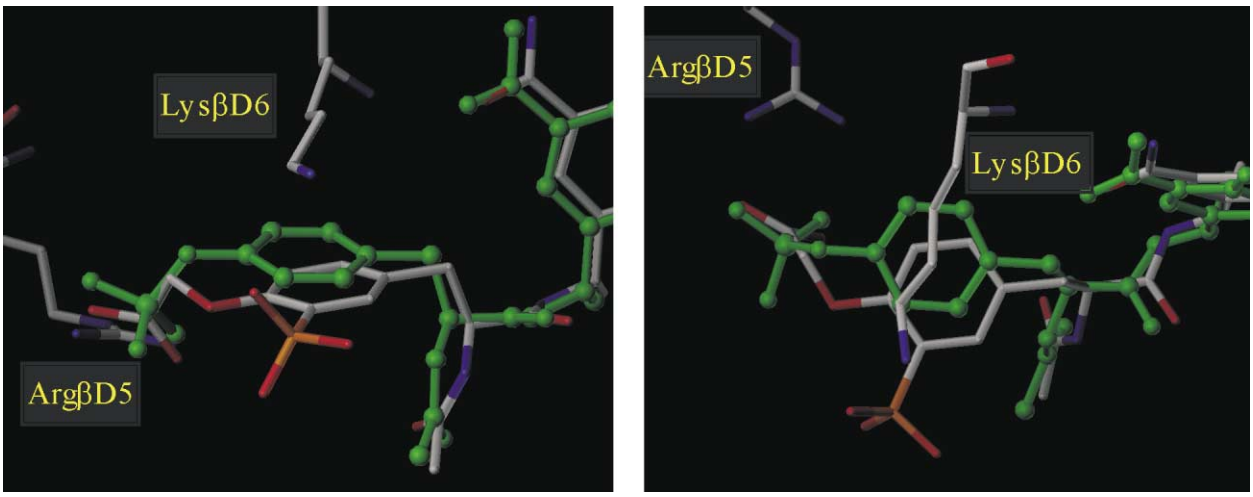


Figure 2. Overlapped representations of **1a** and **1k** bound to a mutant of Lck (119-225) SH2 (Lck119-225 S164C) (X-ray crystallography, 2.2 Å): (a) (left) displays the 'side' view and (b) (right) the 'top' view relative to the plane of the Cpp aromatic ring.

within hydrogen bonding distance to the 3′-phosphonate of the Cpp residue. This may account for the substantial increase in binding affinity of **1a** over **1j** and supports the design concept of this new pTyr mimetic. The relative positions of the benzamide template remain similar between **1a** and **1k**.

In conclusion, the novel pTyr mimetic Cpp has been designed and synthetically incorporated into nonpeptide inhibitors of the Src SH2 domain. X-ray structural studies suggest that both the 4′-carboxymethylether and the 3′-phosphonate groups of the Cpp residue provide effective intermolecular contacts with the Src SH2 pTyr binding pocket. Specifically, the 3′-phosphonate of the Cpp residue dramatically improves binding affinity (cf. **1a** vs **1j**). The binding affinity and cellular activity of **1h** highlight the effectiveness of the monoethyl ester analogue of the Cpp residue in this lead series. These results provide the basis for further studies to exploit the concept of multifunctional group modification of pTyr in the design of SH2 inhibitors.

References and Notes

1. Soriano, P.; Montgomery, C.; Geske, R.; Bradley, A. *Cell* **1991**, *64*, 693.
2. Schwartzberg, P. L.; Xing, L. P.; Hoffmann, O.; Lowell, C. A.; Garrett, L.; Boyce, B.; Varmus, H. *Genes Dev*. **1997**, *11*, 2835.
3. Sawyer, T.; Boyce, B.; Dalgarno, D.; Iuliucci, J. *Expert Opin. Invest. Drugs* **2001**, *10*, 1327.
4. Lutrell, D. K.; Lee, A.; Lansing, T. J.; Crosby, R. M.; Jung, K. D.; Willard, D.; Luther, M.; Rodriguez, M.; Berman, J.; Gilmer, T. M. *Proc. Natl. Acad. Sci. U.S.A*. **1994**, *91*, 83.
5. Cartwright, C. A.; Kamps, M. P.; Miesler, A. I.; Pipas, J. M.; Eckhart, W. *J. Clin. Invest*. **1989**, *83*, 2025.
6. Mazurenko, N. N.; Kogan, E. A.; Zborovskaya, I. B.; Kisseljov, F. L. *Eur. J. Cancer* **1992**, *28*, 372.
7. Thomas, S. M.; Brugge, J. S. *Annu. Rev. Cell. Dev. Biol*. **1997**, *13*, 513.
8. Gilmer, T.; Rodriguez, M.; Jordan, S.; Crosby, R.; Alligood, K.; Green, M.; Kimery, M.; Wagner, C.; Kinder, D.; Charifson, P.; Hassell, A. M.; Willard, D.; Luther, M.; Rusnak, D.; Sternbach, D.; Mehrotra, M.; Peel, M.; Shampine, L.; Davis, R.; Robbins, J.; Patel, I. R.; Kassel, D.; Burkhart, W.; Moyer, M.; Bradshaw, T.; Berman, J. *J. Biol. Chem*. **1994**, *269*, 31711.
9. Lunney, E. A.; Para, K. S.; Rubin, J. R.; Humblet, C.; Fergus, J. H.; Marks, J. S.; Sawyer, T. K. *J. Am. Chem. Soc*. **1997**, *119*, 12471.
10. Sawyer, T. K. *Biopolymers* **1998**, *47*, 243.
11. Burke, T. R., Jr.; Smyth, M. S.; Otaka, A.; Nomizu, M.; Roller, P. P.; Wolf, G.; Case, R.; Shoelson, S. E. *Biochemistry* **1994**, *33*, 6490.
12. Burke, T. R., Jr.; Ye, B.; Akamatsu, M.; Ford, H., Jr.; Yan, X.; Kole, H. K.; Wolf, G.; Shoelson, S. E.; Roller, P. P. *J. Med. Chem*. **1996**, *39*, 1021.
13. Stankovic, C. J.; Surendran, N.; Lunney, E.; Plummer, M. S.; Para, K.; Shahipour, A.; Fergus, J. H.; Marks, J. S.; Herrera, R.; Hubbell, S. E.; Humblet, C.; Saltiel, A. R.; Stewart, B. H.; Sawyer, T. K. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1909.
14. Yao, Z.-J.; King, C. R.; Cao, T.; Kelley, J.; Milne, G.; Voight, J.; Burke, T. R., Jr. *J. Med. Chem*. **1999**, *42*, 23.
15. Beaulieu, P. L.; Cameron, D. R.; Ferland, J.-M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, J. S.; Kabcenell, A.; Krane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. *J. Med. Chem*. **1999**, *42*, 1757.
16. Shakespeare, W. C.; Bohacek, R. S.; Narula, S. S.; Azimioara, M. D.; Yuan, R. W.; Dalgarno, D. C.; Madden, L.; Botfield, M. C.; Holt, D. A. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 3109.
17. Gao, Y.; Luo, J.; Yao, Z.-J.; Guo, R.; Zou, H.; Kelley, J.; Voight, J.; Yang, D.; Burke, T. R., Jr. *J. Med. Chem*. **2000**, *43*, 911.
18. Shakespeare, W.; Yang, M.; Bohacek, R.; Cerasoli, F.; Stebbins, K.; Sundaramoorthi, R.; Azimioara, M.; Vu, C.; Pradeepan, S.; Metcalf, C., III; Haraldson, C.; Merry, T.; Dalgarno, D.; Narula, S.; Hatada, M.; Lu, X.; van Schravendijk, M. R.; Adams, S.; Violette, S.; Smith, J.; Guan, W.; Bartlett, C.; Herson, J.; Iuliucci, J.; Weigele, M.; Sawyer, T. *Proc. Natl. Acad. Sci. U.S.A*. **2000**, *97*, 9373.
19. Casteel, D. A.; Peri, S. P. *Synthesis* **1991**, 691.
20. Kometani, T.; Watt, D. S.; Ji, T. *Tetrahedron Lett*. **1985**, *26*, 2043.
21. Jackson, R. F. W.; Wishart, N.; Wood, A.; James, K.; Wythes, M. J. *J. Org. Chem*. **1992**, *57*, 3397.

22. Luke, G. P.; Holt, D. A. *Tetrahedron: Asymmetry* **1999**, *10*, 4393.
23. Lynch, B. A.; Loiacono, K. A.; Tiong, C. L.; Adams, S. A.; MacNeil, I. A. *Anal. Biochem.* **1997**, *247*, 77.
24. Boyde, A.; Ali, N. N.; Jones, S. J. *Br. Dent. J.* **1984**, *156*, 216.
25. Coordinates have been deposited at the Protein Data Bank. RCSB ID code is RCSB013343 and the PDBID is 1IJR.