Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1199–1202

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Anthranilate Sulfonamide Hydroxamate TACE Inhibitors. Part 2: SAR of the Acetylenic P1′ Group

J. I. Levin,[a,*] J. M. Chen,[a] M. T. Du,[a] F. C. Nelson,[a] L. M. Killar,[b] S. Skala,[b] A. Sung,[b] G. Jin,[a] R. Cowling,[a] D. Barone,[c] C. J. March,[c] K. M. Mohler,[c] R. A. Black[c] and J. S. Skotnicki[a]

[a]*Wyeth-Ayerst Research, 401N. Middletown Road, Pearl River, NY 10965, USA*
[b]*Wyeth-Ayerst Research, PO Box CN-8000, Princeton, NJ 08543, USA*
[c]*Immunex Corporation, Seattle, WA 98101, USA*

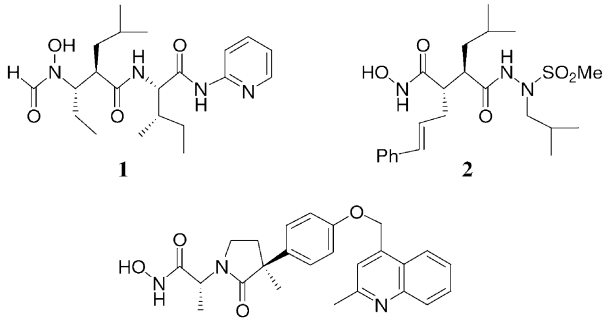
Received 10 December 2001; accepted 4 February 2002

Abstract—The SAR of a series of potent sulfonamide hydroxamate TACE inhibitors bearing novel acetylenic P1′ groups was explored. In particular, compound **4t** bearing a butynyloxy P1′ moiety has excellent in vitro potency against isolated TACE enzyme and in cells, good selectivity over MMP-1 and oral activity in an in vivo model of TNF-α production.

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that exists in two forms, a 26 kDa membrane-bound form and a soluble non-covalently bound homotrimer of 17 kDa units.[1] The enzyme responsible for the cleavage of 26 kDa TNF into soluble TNF is TNF-α converting enzyme (TACE) a member of the ADAM family of enzymes.[2] It has been postulated that agents that inhibit TACE, and thereby reduce levels of soluble TNF-α, might offer an effective treatment for rheumatoid arthritis.[3] To that end a variety of small molecule TACE inhibitors have been reported to date,[3a,4] including the reverse hydroxamates exemplified by **1**,[5] the hydroxamic acid hydrazide **2**,[6] and lactam hydroxamate **3** (Fig. 1).[7]

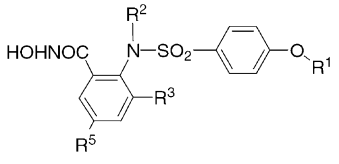
We have previously disclosed several anthranilic acid based sulfonamide hydroxamic acid TACE inhibitors with different MMP inhibition profiles.[8,9] Although the optimization of TACE activity within the anthranilate hydroxamate series led us to several potent inhibitors of this enzyme, including some that were also selective over MMP-1, MMP-9, and MMP-13, none of these compounds had significant (<3 μM) activity as inhibitors of TNF-α production in a THP-1 cellular assay.[10]

In the preceding communication, we have reported the structure-based design and initial discovery of a new class of TACE inhibitors with acetylenic P1′ groups (**4**, Fig. 2, $R^1 = CH_2CCCH_3$).[11] We now wish to report further on the results of our investigation into the SAR of the anthranilate hydroxamic acids in both a TACE enzyme assay and a cellular assay. Compounds have been identified which are highly active against TACE and



1 **2** **3**

Figure 1. Literature TACE inhibitors.



4

Figure 2. Anthranilate hydroxamic acids.

*Corresponding author. Tel.: +1-914-732-3053; fax: +1-914-732-5561; e-mail: levinji@war.wyeth.com

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00136-1

selective over MMP-1, with vastly improved cellular activity. One of these compounds has shown potent inhibitory activity in an in vivo model of TNF-α production.

Chemistry

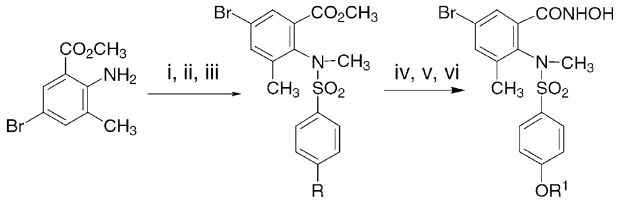
Sulfonamide hydroxamic acids **4a**–**c** (Table 1) were prepared as described previously.[8,9] Compounds **4d**–**k** were prepared from phenol **6b** and the desired propargylic alcohol, as shown in Scheme 1, according to the method described in the preceding paper.[11] Alcohol **4l** was derived in this manner starting from mono-THP protected 2-butyn-1,4-diol.

Piperazine derivative **4t** was also synthesized starting from phenol **6b** as shown in Scheme 2. Protection of the phenol as its TBS-ether (**6c**) was followed by NBS-bromination of the benzylic methyl group. Displacement of the benzylic bromide with 1-methylpiperazine occurred with concomitant desilylation to give phenol **8a**. Mitsunobu alkylation of the phenol with 2-butyn-1-ol and subsequent conversion of the ester into the desired hydroxamic acid via the carboxylic acid gave **4t**. The 3-methylpiperazine-5-phenyl anthranilate derivative, **4u**, was prepared in a similar manner from **7a**, via silyl ether **7b** and piperazine **8b**, with incorporation of the phenyl group accomplished using a Suzuki coupling with phenylboronic acid.

Propargylic ether **4m** and amines **4n**–**p** were prepared from the terminal alkyne **9a**, available from Mitsunobu alkylation of phenol **6b** with propargyl alcohol (Scheme 2). Mannich alkylation with formaldehyde and diethylamine in the presence of CuCl then afforded amine **9b**. Reaction of **9b** with cyanogen bromide then gave the propargylic bromide **9c**. Displacement of the bromide with methanolic sodium hydroxide to give **10a** was followed by conversion of the ester into the hydroxamate as before to give **4m**. Bromide displacement by amines provided **10b**–**d**, which was followed by protection of the resulting propargylic amine with di-*t*-butyl dicarbonate, conversion of the ester into the corresponding hydroxamate and removal of the Boc group to form the hydrochloride salts **4n**–**p**.

Biology

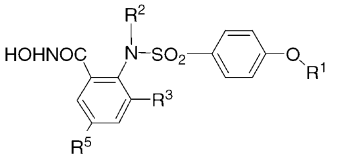
All of the anthranilate hydroxamic acids were tested in vitro[12] for their ability to inhibit MMP-1, MMP-9,



5 **6a** R = F; **6b** R = OH **4d-k**

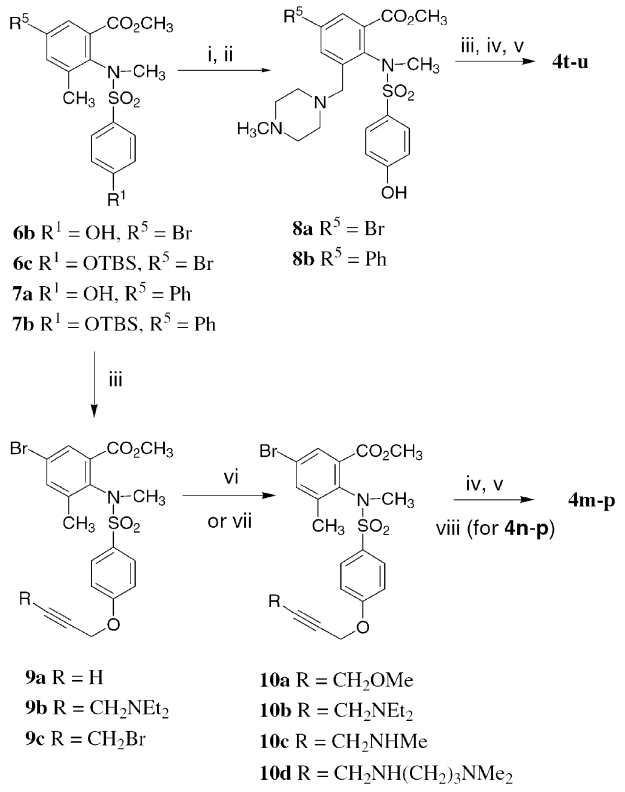
Scheme 1. (i) 4-F-$PhSO_2Cl$, TEA; (ii) CH_3I, K_2CO_3; (iii) (a) CH_3CCCH_2OH, NaH, DMF, 80 °C; (b) HCl, H_2O; (iv) R^1OH, PPh_3, DEAD; (v) NaOH; (vi) (a) $(COCl)_2$, DMF; (b) NH_2OH.

Table 1. In vitro potency of substituted anthranilate hydroxamic acids



Compd	R^1	R^2	R^3	R^5	MMP-1[a]	MMP-9[a]	MMP-13[a]	TACE[a]	THP[b]
4a	CH_3	CH_3	CH_3	Br	114	11	21	32	14
4b	CH_3	CH_3	$CH_2[(CH_2)_2]_2NCH_3$	Br	194	2	5	26	42
4c	CH_2-3-C_5H_4N	CH_3	CH_3	Br	42% (10)	28% (1)	48% (1)	28	9
4d	$(CH_2)_3CH_3$	CH_3	CH_3	Br	2488	21	68	67	0
4e	CH_2CCH	CH_3	CH_3	Br	113	15	52	11	55
4f	$CH(CH_3)CCH$	CH_3	CH_3	Br	1534	455	433	122	9
4g	CH_2CCCH_3	CH_3	CH_3	Br	1616	304	154	16	84
4h	$CH_2CCCH_2CH_3$	CH_3	CH_3	Br	1228	800	289	12	48
4i	$CH_2CC(CH_2)_2CH_3$	CH_3	CH_3	Br	2364	232	358	47	11
4j	$CH_2CC(CH_2)_3CH_3$	CH_3	CH_3	Br	53% (10)	389	701	34	16
4k	CH_2CCPh	CH_3	CH_3	Br	3815	857	321	66	20
4l	CH_2CCCH_2OH	CH_3	CH_3	Br	3203	477	83	7	88
4m	$CH_2CCCH_2OCH_3$	CH_3	CH_3	Br	7803	387	233	11	46
4n	$CH_2CCCH_2NHCH_3$	CH_3	CH_3	Br	26% (10)	643	255	58	15
4o	$CH_2CCCH_2NH(CH_2)_3N(CH_3)_2$	CH_3	CH_3	Br	32% (10)	1205	908	29	14
4p	$CH_2CCCH_2N(CH_2CH_3)_2$	CH_3	CH_3	Br	21% (10)	2827	2377	346	5
4q	CH_2CCCH_3	CH_3	CH_3	H	85% (10)	–	416	28	55
4r	CH_2CCCH_3	H	CH_3	H	6% (10)	–	42% (10)	84	7
4s	CH_2CCCH_3	CH_3	H	H	35% (10)	–	61% (10)	27	32
4t	CH_2CCCH_3	CH_3	$CH_2[(CH_2)_2]_2NCH_3$	Br	1658	166	252	25	94
4u	CH_2CCCH_3	CH_3	$CH_2[(CH_2)_2]_2NCH_3$	Ph	1923	28	47	42	95

[a]IC_{50} (nM) or % inhibition (μM).
[b]% Inhibition at 3 μM.



Scheme 2. (i) NBS; (ii) 1-methylpiperazine, K_2CO_3; (iii) R^1OH, PPh_3, DEAD; (iv) NaOH; (v) (a) $(COCl)_2$, DMF; (b) NH_2OH; (vi) MeOH, NaOH; (vii) excess RNH_2; (viii) HCl(g).

MMP-13 and TACE.[13] Levels of each of these enzymes have been found to be up-regulated in the synovium of RA patients and the broad spectrum inhibition of these enzymes may therefore be therapeutically desirable.[14] However, compounds with selectivity over MMP-1 were also sought in order to gain insight into whether the inhibition of MMP-1 is a possible source of the musculoskeletal side effects seen in clinical trials of broad spectrum MMP inhibitors.[15]

The in vitro potencies for a series of anthranilate hydroxamic acid analogues containing a variety of P1′ groups are shown in Table 1. The simple alkyl ether P1′ groups of compounds **4a**, **4b**, and **4d** provide potent TACE and MMP enzyme inhibition, but little cellular activity. Compounds **4c**–**d**, with lengthier P1′ moieties have reduced potency against MMP-1, due to the shallow S1′ pocket of that enzyme, while TACE activity is minimally diminished. The 3-picolyl derivative, **4c**, is selective for TACE over several MMPs, but again is poorly active in cells at 3 μM.[8]

As we have reported in the preceeding paper, anthranilate hydroxamic acids **4e**, **4g**, and **4j**, bearing acetylenic P1′ groups are also potent inhibitors of TACE enzyme. In addition, compounds **4g** and **4j** are at least 100-fold selective over MMP-1. To our delight, butynyl ether **4g** also possesses a relatively high level of potency in the THP cellular assay, providing 84% inhibition of TNF-α production at 3 μM and 59% inhibition at 1 μM. However, this increased cellular activity was difficult to maintain while modifying the P1′ group. Thus, for the series of linear propargylic ethers **4g–j**, going from a 4-carbon chain to a 7-carbon chain, activity in the THP assay falls off with increasing length of the P1′ group and lipophilicity, although they are all good inhibitors of TACE enzyme (see Table 1). Addition of a branched methyl group on propargyl ether **4e** gives acetylenic ether **4f** and reduces potency against TACE enzyme and in cells. Phenyl acetylene **4k**, in which the distal phenyl ring can extend into the S3′ pocket of TACE, also displays reduced potency against TACE enzyme and in cells relative to butynyl ether **4g**.

Analysis of molecular modeling studies of anthranilate hydroxamates bound to the active site of TACE, making use of the X-ray crystal structure of the TACE catalytic domain,[16] suggested that forming a polar interaction between the ligand and the glutamate residue residing in the S3′ pocket of TACE offered an opportunity for further enhancing binding potency and selectivity. To that end the propargylic alcohol **4l**, ether **4m**, and amines **4n–p** were prepared. Alcohol **4l** proved to be the most potent inhibitor of TACE in the anthranilate series. Compound **4l** also offered selectivity over both MMP-1 and MMP-9 and cellular activity equal to butynyl ether **4g**. Compound **4l** gave 69% inhibition of TNF-α production at a concentration of 1 μM. The analogous methyl ether, **4m**, has substantially the same MMP/TACE profile as alcohol **4l**, but with diminished cellular activity. Amine **4n** is 5-fold less potent against TACE than the isosteric methyl ether **4m**, and has much weaker potency in cells. Diamine **4o** is the most potent and selective TACE inhibitor of the propargylic amines, with a selectivity profile similar to the picolyl analogue **4c**. Unfortunately, as with **4c**, diamine **4o** has only very low level cellular activity. The more sterically demanding diethylamine, **4p**, is still weaker in cells as well as versus the MMPs and TACE enzyme.

The search for compounds selective for TACE over MMP-1 and MMP-13 was also addressed by paring down functionality from the anthranilate hydroxamic acids that had been required for potency against the MMPs and TACE prior to the utilization of the acetylenic P1′ groups. For example, removal of the 5-bromo substituent from butynyl analogue **4g** to give **4q** does not appreciably affect MMP/TACE activity. Compound **4r**, the NH–sulfonamide analogue of **4q** is less potent than **4q** against TACE and MMP-1 and MMP-13. Elimination of both the 5-bromo and 3-methyl substituents from **4g** to provide **4s** does not reduce TACE enzyme activity at all, but does abolish activity against MMP-1 and MMP-13 to a great extent resulting in a very selective TACE inhibitor. Each of these analogues unfortunately suffers from diminished cellular activity relative to parent compound **4g**.

Finally, since previous work[8] on the anthranilate hydroxamates had shown that oral activity is greatly enhanced by the incorporation of a basic amine moiety into the inhibitor molecule, we targeted analogues of piperazine **4b** which would also contain the P1′ butynyl moiety. The resulting compounds, **4t** and **4u**, are the

most potent anthranilate hydroxamic acids in the THP cellular assay and have good potency against TACE enzyme. Both **4t** and **4u** provide slightly greater than 50% inhibition at 300 nM in THP-1 cells.

Due to the excellent potency of compound **4t** in the TACE enzyme and THP cellular assays it was tested in a preliminary in vivo model to measure its ability to inhibit the LPS stimulated production of TNF-α in a mouse.[10] A 50 mg/kg oral dose of compound **4t** provided 100% inhibition of TNF-α production 1 h after dosing. The effect lasted at least 6 h post dosing, with a 75% inhibition of TNF-α production at that time point.

In summary, we have synthesized a series of anthranilate-hydroxamic acid inhibitors of MMPs and TACE with novel propargylic ether P1′ substituents. Many of these compounds are potent inhibitors of TACE in an isolated enzyme assay. Several of these compounds (**4g**–**h**, **4j**, and **4l**–**o**) are greater than 100-fold selective for TACE over MMP-1. In an assay measuring the inhibition of TNF-α production in THP-1 cells four members of the butynyl P1′ series of analogues (**4g**, **4l**, and **4t**–**u**) produced greater than 50% inhibition at concentrations of 1 μM or less. Most importantly, compound **4t** has been shown to inhibit TNF-α production with good duration of action on oral dosing in an in vivo murine LPS model. The application of acetylenic P1′ groups to other TACE inhibitor scaffolds will be reported in due course.

Acknowledgements

J.I.L. dedicates this manuscript to the memory of Marvin H. Levin.

References and Notes

1. (a) Aggarwal, B. B.; Kohr, W. J.; Hass, P. E.; Moffat, B.; Spencer, S. A.; Henzel, W. J.; Bringman, T. S.; Nedwin, G. E.; Goeddel, D. V.; Harkins, R. N. *J. Biol. Chem.* **1985**, *260*, 2345. (b) Tracey, K. J.; Cerami, A. *Annu. Rev. Med.* **1994**, *45*, 491. (c) Aggarwal, B. B.; Natarajan, K. *Euro. Cytokine Net.* **1996**, *7*, 93. (d) Bemelmans, M. H. A.; van Tits, L. J. H.; Buurman, W. A. *Crit. Rev. Immunol.* **1996**, *16*, 1.

2. (a) Moss, M.; Becherer, J. D.; Milla, M.; Pahel, G.; Lambert, M.; Andrews, R.; Frye, S.; Haffner, C.; Cowan, D.; Maloney, P.; Dixon, E. P.; Jansen, M.; Vitek, M. P.; Mitchell, J.; Leesnitzer, T.; Warner, J.; Conway, J.; Bickett, D. M.; Bird, M.; Priest, R.; Reinhard, J.; Lin, P. In *Metalloproteinases as Targets for Anti-inflammatory Drugs*; Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds.; Birkhauser: Basel, 1999; p 187. (b) Killar, L.; White, J.; Black, R.; Peschon, J. *Ann. N. Y. Acad. Sci.* **1999**, *878*, 442.

3. (a) Nelson, F. C.; Zask, A. *Exp. Opin. Invest. Drugs* **1999**, *8*, 383. (b) Lowe, C. *Exp. Opin. Ther. Pat.* **1998**, *8*, 1309. (c) Newton, R. C.; Decicco, C. P. *J. Med. Chem.* **1999**, *42*, 2295.

4. Moss, M. L.; White, J. M.; Lambert, M. H.; Andrews, R. C. *Drug Discov. Today* **2001**, *6*, 417.

5. (a) Musso, D. L.; Andersen, M. W.; Andrews, R. C.; Austin, R.; Beaudet, E. J.; Becherer, J. D.; Bubacz, D. G.; Bickett, D. M.; Chan, J. H.; Conway, J. G.; Cowan, D. J.; Gaul, M. D.; Glennon, K. C.; Hedeen, K. M.; Lambert, M. H.; Leesnitzer, M. A.; McDougald, D. L.; Mitchell, J. L.; Moss, M. L.; Rabinowitz, M. H.; Rizzolio, M. C.; Schaller, L. T.; Stanford, J. B.; Tippin, T. K.; Warner, J. R.; Whitesell, L. G.; Wiethe, R. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2147. (b) Rabinowitz, M. H.; Andrews, R. C.; Becherer, J. D.; Bickett, D. M.; Bubacz, D. G.; Conway, J. G.; Cowan, D. J.; Gaul, M.; Glennon, K.; Lambert, M. H.; Leesnitzer, M. A.; McDougald, D. L.; Moss, M. L.; Musso, D. L.; Rizzolio, M. C. *J. Med. Chem.* **2001**, *44*, 4252.

6. (a) Broadhurst, M. J.; Johnson, W. H.; Walter, D. S. PCT Int. Appl. WO0032570, 2000; *Chem. Abstr.* **2000**, *133*, 30513. (b) *Exp. Opin. Ther. Pat.* **2000**, *10*, 1617.

7. (a) Duan, J.; Decicco, C. P.; Wasserman, Z. R.; Maduskuie, T. P., Jr. PCT Int. Appl., WO9918074, 1999; *Chem. Abstr.* 1999, *130*, 296611. (b) Decicco, C. *Abstracts of Papers*, 221st National Meeting of the American Chemical Society, San Diego, CA, April 1–5, 2001; American Chemical Society: Washington, DC, **2001**; MEDI 189.

8. Levin, J. I.; Chen, J.; Du, M.; Hogan, M.; Kincaid, S.; Nelson, F.; Venkatesan, A. M.; Wehr, T.; Zask, A.; DiJoseph, J.; Killar, L. M.; Skala, S.; Sung, A.; Sharr, M.; Roth, C.; Jin, G.; Cowling, R.; Mohler, K. M.; Black, R. A.; March, C. J.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2189.

9. Levin, J. I.; Chen, J. M.; Du, M. T.; Nelson, F. C.; Wehr, T.; DiJoseph, J. F.; Killar, L. M.; Skala, S.; Sung, A.; Sharr, M. A.; Roth, C. E.; Jin, G.; Cowling, R.; Di, L.; Sherman, M.; Xu, Z. B.; March, C. J.; Mohler, K. M.; Black, R. A.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2975.

10. (a) Mohler, K. M.; Sleath, P. R.; Fitzner, J. N.; Cerretti, D. P.; Alderson, M.; Kerwar, S. S.; Torrance, D. S.; Otten-Evans, C.; Greenstreet, T.; Weerawarna, K.; Kronheim, S. R.; Petersen, M.; Gerhart, M.; Kozlosky, C. J.; March, C. J.; Black, R. A. *Nature* **1994**, *370*, 218. (b) Levin, J. I.; Chen, J. M.; Cole, D. C. WO 00/44709, 2000; *Chem. Abstr.* **2000**, *133*, 150908.

11. Chen, J. M.; Jin, G.; Sung, A.; Levin, J. I. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1195.

12. (a) Weingarten, H.; Feder, J. *Anal. Biochem.* **1985**, *147*, 437.
(b) Inhibitor concentrations were run in triplicate. MMP IC_{50} determinations were calculated from a four-parameter logistic fit of the data within a single experiment.

13. Jin, G.; Black, R.; Wolfson, M.; Rauch, C.; Ellestad, G. A.; Cowling, R., *Anal. Biochem.* **2002**, *302*, 269.

14. (a) Yoshihara, Y.; Nakamura, H.; Obata, K.; Yamada, H.; Hayakawa, T.; Fujikawa, K.; Okada, Y. *Ann. Rheum. Dis.* **2000**, *59*, 455. (b) Konttinen, Y. T.; Ainola, M.; Valleala, H.; Ma, J.; Ida, H.; Mandelin, J.; Kinne, R. W.; Santavirta, S.; Sorsa, T.; Lopez-Otin, C.; Takagi, M. *Ann. Rheum. Dis.* **1999**, *58*, 691.

15. Scrip **1998**, *2349*, 20.

16. Maskos, K.; Fernandez-Catalan, C.; Huber, R.; Bourenkov, G. P.; Bartunik, H.; Ellestad, G. A.; Reddy, P.; Wolfson, M. F.; Rauch, C. T.; Castner, B. J.; Davis, R.; Clarke, H. R. G.; Petersen, M.; Fitzner, J. N.; Cerreti, D. P.; March, C. J.; Paxton, R. J.; Black, R. A.; Bode, W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3408.