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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3966–3970

# The synthesis and in vitro testing of structurally novel antibiotics derived from acylnitroso Diels–Alder adducts

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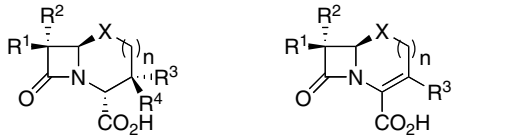
Received 17 March 2006; revised 5 May 2006; accepted 5 May 2006
Available online 26 May 2006

**Abstract**—The structural similarity between β-lactam antibiotics, such as penicillin, and isoxazolidine-3,5-dicarboxylic acids led to the hypothesis that isoxazolidine-3,5-dicarboxylic acids could be effective analogs of β-lactam antibiotics. The syntheses of relevant isoxazolidine-3,5-dicarboxylic acids from acylnitroso Diels–Alder adducts and subsequent biological testing have shown that these first examples are inhibitors of *Escherichia coli* X580.


Bacterial resistance to antibiotics has driven the search for new, more effective antibacterial agents.[1] New analogs of β-lactam antibiotics are some of the most sought after compounds due to their expected low toxicity in humans and potential broad spectrum application. Extensive structure–activity relationship (SAR) work has revealed a number of analogs of penicillin that have clinically relevant levels of antibacterial activity. This effort has been concentrated on altering the ring size, peripheral substituents (R groups), and heteroatoms (X = S, O, and $CH_2$) on the bicyclic ring system common to β-lactam antibiotics (Fig. 1).[2]

In addition to these classical alterations, increasing the number of rings has been tried with some promising success.[3] However, this increases the length and complexity of the syntheses of these molecules compared to bicyclic β-lactams. So from a synthetic point of view a smaller molecule would be desired. Ideally a monocyclic β-lactam would fit this criterion, but the development of effective monocyclic β-lactams was hampered by the belief that monocyclic β-lactams would not be effective antibacterial agents because loss of the bicyclic system would decrease the electrophilicity of the β-lactam carbonyl. This idea was reinforced by the discovery of the nocardicins,[4] which are natural monocyclic β-lactams that do not have significant antibiotic activity (Fig. 2). Attachment of electron-withdrawing groups to the monocyclic β-lactam has alleviated the problem of the electrophilicity of the carbonyl. The best examples of heteroatom-activated monocyclic β-lactams are the oxamazins,[5] monobactams,[6] and monosulfactams.[7] The thiamazins are further proof that activation of the carbonyl requires a highly electron-withdrawing group, since they are not active. Though this may also be due to the thiamazins' N–S bond being longer than the N–O bond of the oxamazins and thus not fitting into the active site.[5d]

Since the bicyclic system of β-lactam antibiotics is not needed, the next logical question is if the β-lactam ring is required for activity. This question has been explored but has yet to produce a compound with significant antibacterial activity.[8] It came to our attention that isoxazolidines **1**, developed from acylnitroso Diels–Alder




penam (X=S, n=0)
cepham (X=S, n=1)
carbacepham (X=$CH_2$, n=1)
oxacepham (X=O, n=1)

penem (X=S, n=0)
cephem (X=S, n=1)
carbacephem (X=$CH_2$, n=1)
oxacephem (X=O, n=1)
oxapenem (X=O, n=0)

**Figure 1.** Bicyclic β-lactam antibiotics.

*Keywords*: β-Lactam antibiotics; Isoxazolidine-3,5-dicarboxylic acids; Antibiotic analogs; Acylnitroso cycloaddition; Hetero Diels–Alder; Oxamazin and monobactam analogs.
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doi:10.1016/j.bmcl.2006.05.021

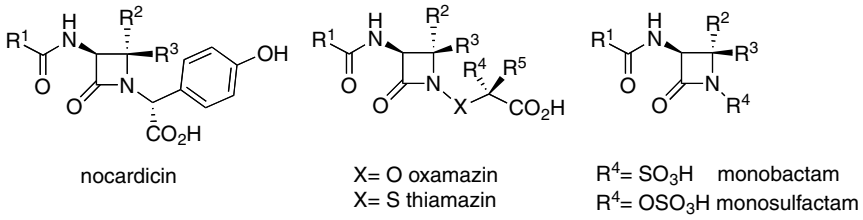



**Figure 2.** Monocyclic β-lactam antibiotics.

adducts[9] **2**, contained all the necessary functionality that is known to be needed for activity, and show structural similarity to the acyl-D-Ala-D-Ala (Fig. 3).[10] In addition, like the oxamazins and monosulfactams, it is possible that oxygen attached to the nitrogen in **1** might make the carbonyl electrophilic enough to be attacked like the β-lactam carbonyl.

In order to test this hypothesis, three isoxazolidines were synthesized and tested against bacteria. The synthesis of the desired isoxazolidines began by acylation of glycine,

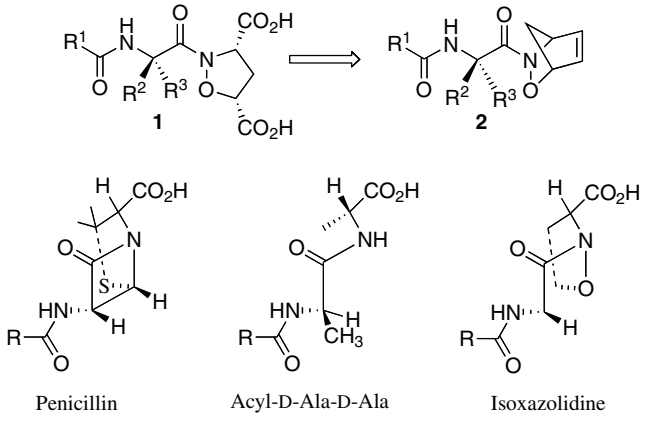
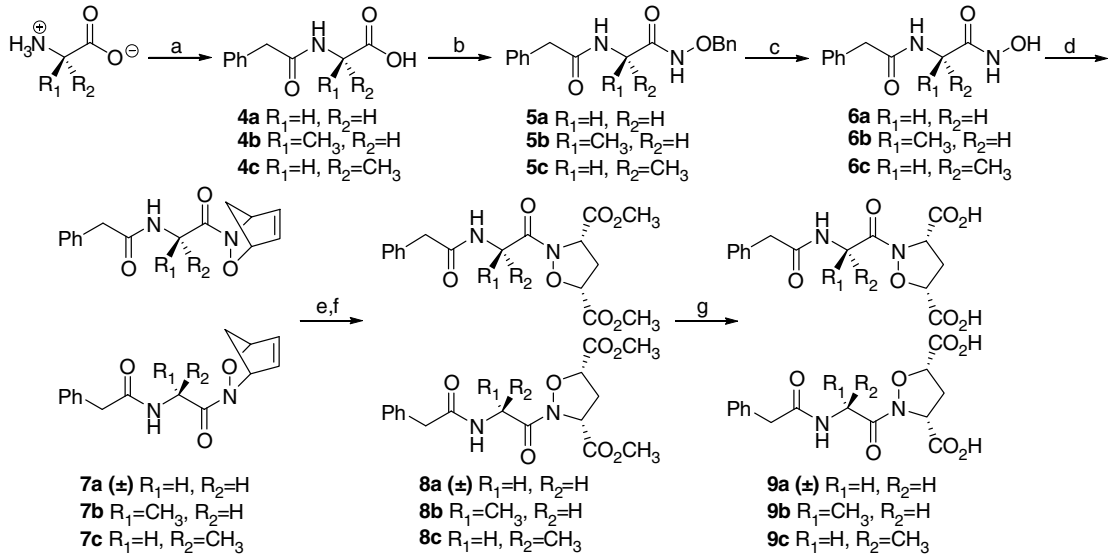



**Figure 3.** Structural comparison of isoxazolidines to penicillin.

D-alanine, and L-alanine[11] followed by coupling with *O*-benzylhydroxylamine (OBHA) to give hydroxamates **5a**–**c** (Scheme 1). Hydrogenation of **5a**–**c** gave hydroxamic acids **6a**–**c**. Oxidation with sodium periodate generated the transient acylnitroso moieties which reacted with freshly distilled cyclopentadiene (cp) to give cycloadducts **7a**–**c** as mixtures of enantiomers (for **7a**) and diastereomers (for **7b** and **7c**) which were carried through the rest of the syntheses. Syntheses of hydroxamic acids **6a**–**c** were also achieved by treatment of the methyl esters of **4a**–**c** with alkaline hydroxylamine. The isoxazolidine dimethyl esters **8a**–**c** were obtained by oxidative cleavage[12] of cycloadducts **7a**–**c** followed by treatment with diazomethane to facilitate isolation and purification. Saponification of **8a**–**c** gave the desired isoxazolidines **9a**–**c**.

Isoxazolidines **9a**–**c** were tested against *Escherichia coli* X580,[13,14] a strain of bacteria that is hypersensitive to β-lactam compounds, and isoxazolidines **9a**–**c** showed promising activity. As can be seen from the kinetic growth curves below (Figs. 4 and 5), both **9a**–**b** inhibit the growth of *E. coli* X580 compared to a control containing DMSO and *E. coli* X580.

Additional antibacterial testing was conducted using an agar diffusion assay. The data from this extended study again show that isoxazolidines **9a**–**c** are active against *E. coli* X580, but as expected, not as active




**Scheme 1.** Reagents and conditions: (a) phenylacetyl chloride, $CH_2Cl_2$, NaOH, $H_2O$; (b) OBHA, EDC, THF, $H_2O$, pH 4.5; (c) Pd on carbon, $H_2$, $CH_3OH$; (d) cp, $NaIO_4$, $CH_3OH$, $H_2O$; (e) $RuCl_3$, $NaIO_4$, $CH_3CN$, $H_2O$, $CCl_4$; (f) $N_2CH_2$, ether; (g) LiOH, THF, $H_2O$.

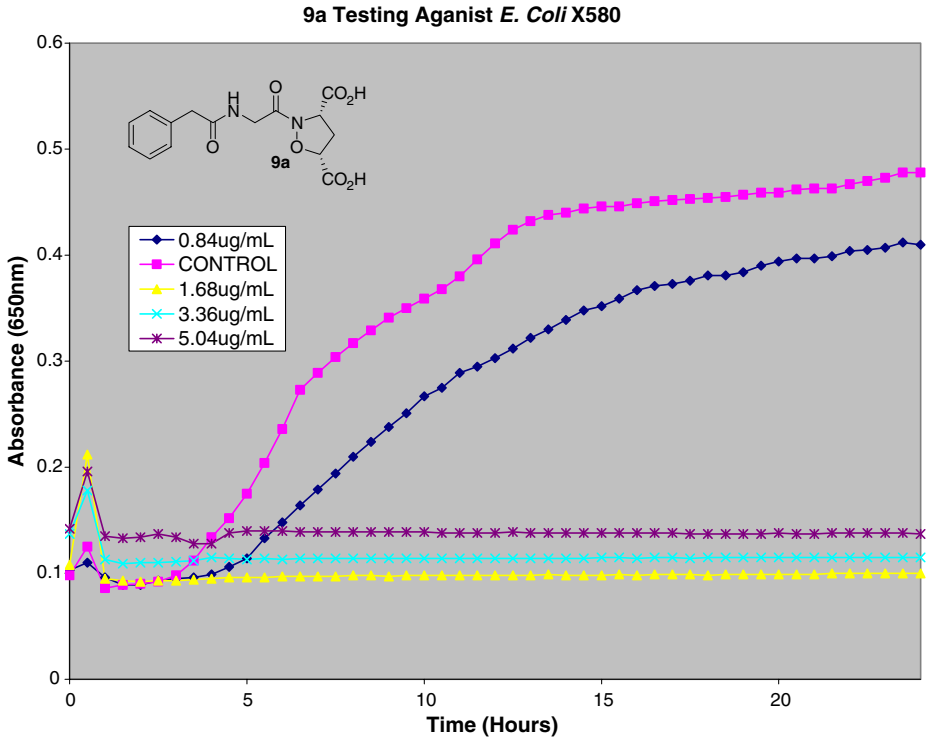



**Figure 4.** Testing of 9a against E. Coli X580.

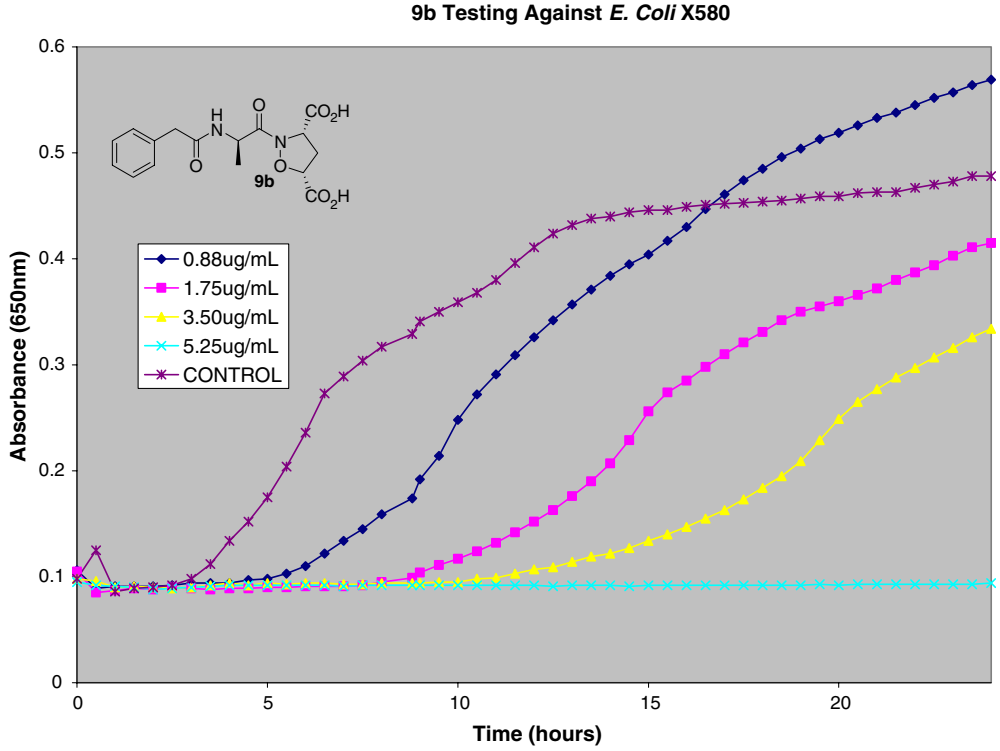



**Figure 5.** Testing of 9b against E. Coli X580.

as penicillin G (Table 1). These data also directly show that *E. coli* X580 is much more susceptible to penicillin G compared to *E. coli* ATCC 33475. However, when isoxazolidines **9a**–**c** were tested against several clinically relevant strains of bacteria, they were found to be devoid of activity, whereas several of the same strains were susceptible to other antibiotics. Again, these results parallel those of earlier studies

**Table 1.** Antimicrobial activity in the agar diffusion assay Growth inhibition zones in mm

| Species | Strain | Relevant property | **9a** | **9b** | **9c** | Penicillin G | Lorabid | Cefotaxime | Ciprofloxacin 5 μg/ml |
|---|---|---|---|---|---|---|---|---|---|
| *Gram + bacteria* | | | | | | | | | |
| *Staphylococcus aureus* | SG 511 | Wild type | 0 | NA | NA | NA | 28 | 26 | 29 |
| *Staphylococcus aureus* | 134/93 | MRSA | 0 | NA | NA | NA | 0 | 0 | 0 |
| *Enterococcus faecalis* | 1528 | VRE | 0 | NA | NA | NA | 0 | 22 | 24 |
| *Mycobacterium vaccae* | IMET 10670 | Wild type | 0 | NA | NA | NA | 0 | 0 | 38 |
| *Gram − bacteria* | | | | | | | | | |
| *Escherichia coli* | DC0 | Wild type | 0 | NA | NA | NA | 24 | 21 | 25 |
| *Escherichia coli* | IV-3-2 | TEM1 β-lactamase | 0 | NA | NA | NA | 23 | 28 | 27 |
| *Enterobacter cloacae* | P99 | ampC β-lactamase | 0 | NA | NA | NA | 0 | 0 | 30 |
| *Pseudomonas aeruginosa* | IV-3-13 | PSE1 β-lactamase | 0 | NA | NA | NA | 0 | 14p | 35 |
| *Escherichia coli* | X580 | NA | 26 | 25 | 20 | 54 | NA | NA | NA |
| *Escherichia coli* | ATCC 33475 | NA | 0 | 0 | 0 | 23 | NA | NA | NA |
| *Samonella enterica* | ATCC 13311 | NA | 0 | 0 | 0 | 37 | NA | NA | NA |
| *Klebsiella pneumoniae* | ATCC 8308 | NA | 0 | 0 | 0 | 12 | NA | NA | NA |
| *Fungus* | | | | | | | | | |
| *Candida albicans* | BMSY 212 | NA | 0 | NA | NA | NA | NA | NA | NA |

NA, not acquired.
p: colonies within the inhibition zone.
Test organisms ($10^6$ CFU/ml) were suspended in melted Nutrient agar (Serva) and poured into Petri dishes. Holes of 9 mm in diameter were made in the agar and filled with 50 μl of a 0.2 mM solution of the compounds. Inhibition zones for bacteria were read after incubation for 18 h at 37 °C, for *Candida albicans* at 30 °C.

with oxamazins.[5] A representative isoxazolidine, **9c**, was also tested for β-lactamase inhibitory activity using penicillinase from *Bacillus cereus* (EC 3.5.2.6) under standard conditions[15] and was found not to be an inhibitor.

If isoxazolidines **9a–c** are acting through the same pathway that β-lactam antibiotics use, then, as with the previously studied oxamazins[5] and other monobactams,[6,7] alteration of the phenylacetyl side chain would be expected to improve broad spectrum activity. Efforts are continuing in our laboratory to increase and broaden the activity of these and related isoxazolidines.

## Acknowledgments

Special thanks go to Anna Riley, Doug Zeckner, and Eli Lilly and Company for broad screen testing of our compounds. We gratefully acknowledge the NIH (GM068012) for partial support of this research.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.05.021.

## References and notes

1. (a) *Multiple Drug Resistant Bacteria*; Amábile-Cuevas, C. F., Ed.; Horizon Scientific Press: England, 2003; pp 1–182; (b) Weissman, K. *Chem. Br.* **2002**, *39*, 22.
2. (a) *The Chemistry of Penicillin*; Clarke, H. T., Johnson, J. R., Robinson, S. R., Eds.; Princeton University Press: Princeton, 1949; (b) *Chemistry and Biology of β-Lactam Antibiotics Penicillins and Cephalosporins*; Morin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; Vol. 1, (c) *Chemistry and Biology of β-Lactam Antibiotics Nontraditional β-Lactam Antibiotics*; Morin, R. B., Gorman, M., Eds.; Academic Press: New York, 1982; Vol. 2.
3. (a) Niu, C.; Pettersson, T.; Miller, M. J. *J. Org. Chem.* **1996**, *61*, 1014; (b) Duboc, R.; Hénaunt, C.; Savignac, M.; Genet, J.; Bhatnagar, N. *Tetrahedron Lett.* **2001**, *42*, 2461; (c) Rossi, T.; Biondi, S.; Contini, S.; Thomas, R. J.; Marchioro, C. *J. Am. Chem. Soc.* **1995**, *117*, 9604; (d) Andreotti, D.; Biondi, S.; Fabio, R. D.; Donati, D.; Piga, E.; Rossi, T. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2019; (e) Andreotti, D.; Biondi, S.; Donati, D.; Lociuro, S.; Pain, G. *Can. J. Chem.* **2000**, *78*, 772; (f) Furman, B.; Molotov, S.; Thürmer, R.; Kaluza, Z.; Voelter, W.; Chmielewski, M. *Tetrahedron* **1997**, *53*, 5883.
4. (a) Isenring, H. P.; Hofheinz, W. *Tetrahedron* **1983**, *39*, 2591; (b) Kamiya, T.; Hashimoto, M.; Nakaguchi, O.; Oku, T. *Tetrahedron* **1979**, *35*, 323; (c) Nishida, M.; Mine, Y.; Nonoyama, S.; Kojo, H.; Goto, S.; Kuwahara, S. *J. Antibiot.* **1977**, *30*, 917; (d) Hashimoto, M.; Komori, T.; Kamiya, T. *J. Am. Chem. Soc.* **1976**, *98*, 3023.
5. (a) Ghosh, M.; Miller, M. J. *Tetrahedron* **1996**, *52*, 4225; (b) Zercher, C. K. *Diss. Abstr. Int. B* **1990**, *50*, 3485; (c) Guanti, G.; Baldaro, E.; Banfi, L.; Guaragna, A.; Narisano, E.; Valcavi, U. *Tetrahedron* **1988**, *44*, 3685; (d) Boyd, D. B.; Eigenbrot, C.; Indelicato, J. M.; Miller, M. J.; Pasini, C. E.; Woulfe, S. R. *J. Med. Chem.* **1987**, *30*, 528; (e) Woulfe, S. R.; Miller, M. J. *J. Org. Chem.* **1986**, *51*, 3133; (f) Woulfe, S. R.; Miller, M. J. *Tetrahedron Lett.* **1984**, *25*, 3293; (g) Woulfe, S. R.; Miller, M. J. *J. Med. Chem.* **1985**, *28*, 1447.
6. (a) Sykes, R. B.; Bonner, D. P.; Bush, K.; Georgopapadakou, N. H. *Antimicrob. Agents Chemother.* **1982**, *21*, 85; (b) Sykes, R. B.; Cimarusti, C. M.; Bonner, D. P.; Bush, K.; Floyd, D. M.; Georgopapadakou, N. H.; Koster, W. H.; Liu, W. C.; Parker, W. L.; Principle, P. A.; Rathnum, M. L.; Slusarchyk, W. A.; Trejo, W. H.; Wells, J. S. *Nature* **1981**, *291*, 489; O'Sullivan, J.; Gillum, A. M.; Aklonis, C. A.; Souser, M. L.; Sykes, R. B. *Antimicrob. Agents Chemother.* **1982**, *21*, 558.

7. (a) Gordon, E. M.; Ondetti, M. A.; Pluscec, J.; Cimarusti, C. M.; Bonner, D. P.; Sykes, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 6053; (b) Cimarusti, C. M.; Sykes, R. B. *Med. Res. Rev.* **1984**, *4*, 1.
8. Boyd, D. B.; Foster, B. J.; Hatfield, L. D.; Hornback, W. J.; Jones, N. D.; Munroe, J. E.; Swartzendruber, J. K. *Tetrahedron Lett.* **1986**, *27*, 3457.
9. (a) Ghosh, A.; Miller, M. J. *Tetrahedron Lett.* **1995**, *36*, 6399; (b) Ritter, A. R.; Miller, M. J. *Tetrahedron Lett.* **1994**, *35*, 9379; (c) Vogt, P. F.; Miller, M. J. *Tetrahedron* **1998**, *54*, 1317; (d) Hansel, J.-G.; O'Hogan, S.; Lensky, S.; Ritter, A. R.; Miller, M. J. *Tetrahedron Lett.* **1995**, *36*, 2913.
10. One carboxyl group on the isoxazolidine was removed for clarity.
11. Hitchcock, S. R.; Nora, G. P.; Hedberg, C.; Casper, D. M.; Buchanan, L. S.; Squire, M. D.; West, D. X. *Tetrahedron* **2000**, *56*, 8799.
12. (a) Carlsen, H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936; (b) Shireman, B. T.; Miller, M. J.; Jonas, M.; Wiest, O. *J. Org. Chem.* **2001**, *66*, 6046.
13. (a) Dolence, E. K.; Minnick, A. A.; Miller, M. J. *J. Med. Chem.* **1990**, *33*, 461; (b) Ramurthy, S.; Miller, M. J. *J. Org. Chem.* **1996**, *61*, 4120.
14. A similar method of antibacterial testing Lu, Y.; Miller, M. J. *Bioorg. Med. Chem.* **1999**, *7*, 3025.
15. Penicillinase from *Bacillus cereus* (EC 3/5/2/6) was obtained from SIGMA (product P 0389) and the assay was performed according to the supplied procedure.