*[6-chloro-3-pyridylmethyl-^{3}H]*Neonicotinoids as High-Affinity Radioligands for the Nicotinic Acetylcholine Receptor: Preparation Using NaB^3H_4 and LiB^3H_4

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SUMMARY

NaB^3H_4 and LiB^3H_4 at 78% and 97% isotopic enrichments, respectively, were used in the synthesis of ^{3}H-labeled 1-(6-chloro-3-pyridyl)-methyl-2-nitromethyleneimidazolidine (CH-IMI) and *N'*-[(6-chloro-3-pyridyl)methyl]-*N''*-cyano-*N'*-methylacetamidine (acetamiprid) (two very potent insecticides) and of 1-(6-chloro-3-pyridyl)methyl-2-iminoimidazolidine (desnitro-IMI) (a metabolite of the commercial insecticide imidacloprid). 6-Chloronicotinoyl chloride was treated with either NaB^3H_4 in methanol or LiB^3H_4 in tetrahydrofuran and the resulting alcohol transformed to 2-chloro-5-chloromethylpyridine, which was then coupled to *N*-cyano-*N'*-methylacetamidine to give [^{3}H]acetamiprid (45 Ci/mmol). 2-Chloro-5-chloro[^{3}H]methylpyridine was also reacted with ethylenediamine and the product was either refluxed in absolute ethanol with 1,1-bis(methylthio)-2-nitroethylene to provide [^{3}H]CH-IMI or reacted in toluene with a solution of cyanogen bromide to produce [^{3}H]desnitro-IMI (each 55 Ci/mmol).

Key words: imidacloprid analogs, insecticide, lithium borotritide, nicotinic acetylcholine receptor, radioligand, sodium borotritide, tritium-labelling

INTRODUCTION

Neonicotinoids, acting at the nicotine binding site of the nicotinic acetylcholine receptor (nAChR), are a major new class of insecticides optimized over the past 24 years (Figure 1)[1,2]. The lead compound was a dibromonitromethane derivative[3] observed to have insecticidal activity. It was modified to produce the nitromethylene analog designated nithiazine, which has outstanding potency as a systemic insecticide[4] but unacceptable photolability.[5,6] Introduction of the chloropyridylmethyl moiety greatly increased the potency in the optimized

CCC 0362–4803/96/110971–08

Received 11 March 1996
Revised 10 June 1996

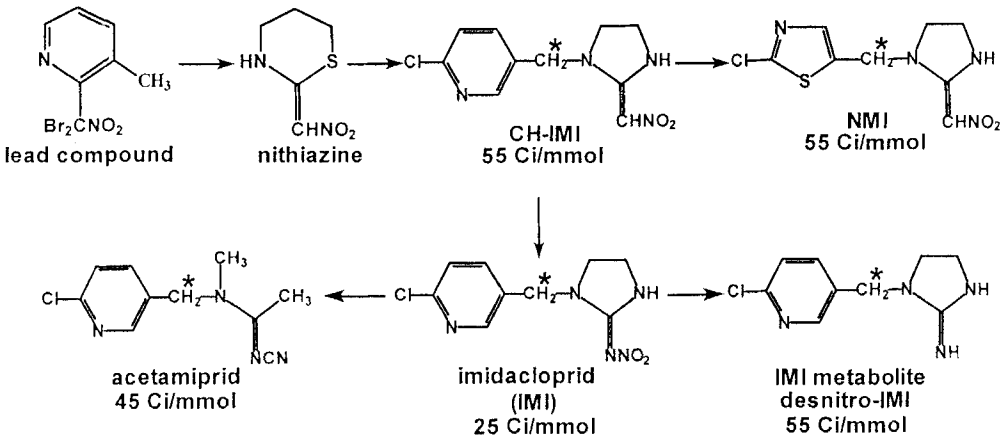


Figure 1. Phylogeny of chloropyridylmethyl and chlorothiazolylmethyl neonicotinoids as insecticides or a metabolite (desnitro-IMI). Asterisks indicate the positions of incorporated tritium in the radioligands. Their specific activities are also shown.

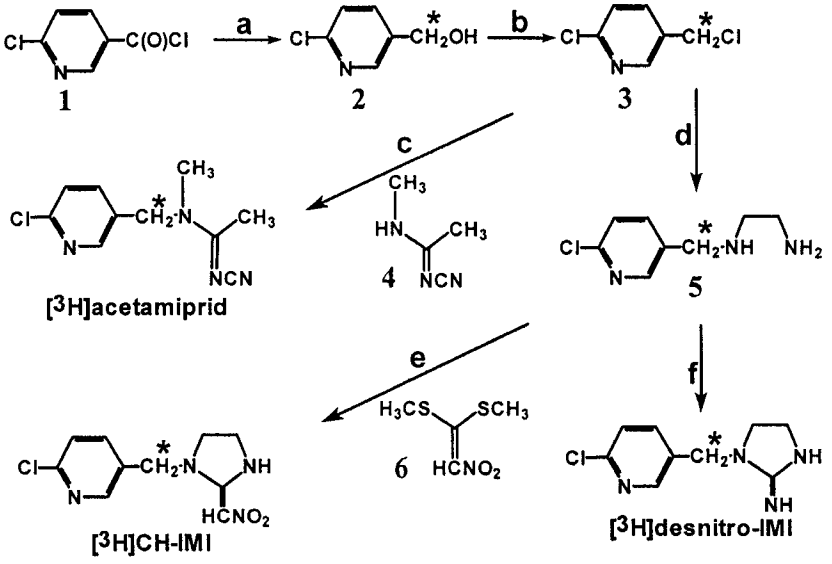
1-(6-chloro-3-pyridyl)methyl-2-nitromethyleneimidazolidine (CH-IMI).[7] The instability of this compound when exposed to light was overcome by substituting the nitromethylene by a nitroimine to give imidacloprid (IMI)[8,9] which was the first commercial member of the new class of neonicotinoids. Desnitro-IMI is also of interest as one of the initial metabolites of IMI in plants, animals and soils.[8,10] The analog acetamiprid has broad spectrum activity including control of lepidopterous larvae and white flies.[11-13]

Synthesis of [^{3}H]IMI with a specific activity of 25 Ci/mmol[14] provided an outstanding radioligand that undergoes high-affinity specific binding to the nAChR of the central nervous system in house fly head membranes.[15] 1-(2-Chloro-5-thiazolyl)methyl-2-nitromethyleneimidazolidine (NMI), which is 6-fold more effective than IMI in displacing [^{3}H]IMI from its binding site in the house fly nAChR,[16] was prepared from $LiAl^3H_4$[17] giving a specific activity close to the theoretical value. In the present study we report the preparation of [^{3}H]CH-IMI, [^{3}H]desnitro-IMI and [^{3}H]acetamiprid using NaB^3H_4[18] and LiB^3H_4 as the source of tritium in the radiosynthesis.

RESULTS and DISCUSSION

The syntheses were based on the route developed for the preparation of [^{3}H]IMI.[14] NaB^3H_4 at an isotopic enrichment of 78% was prepared by hydrogen-tritium exchange at 450 °C for 6 hr.[18] LiB^3H_4 can also be prepared by this procedure at 270 °C with an isotopic enrichment of 73%; at higher temperature (300 °C) this borohydride was decomposed.[18] However, LiB^3H_4 at an isotopic enrichment of 97% can be achieved using a procedure similar to the one developed for the synthesis of $LiAl^3H_4$.[17]

Thus to prepare [^{3}H]acetamiprid (see Scheme 1), 6-chloronicotinoyl chloride (**1**) was reduced in methanol using NaB^3H_4 to [^{3}H]**2** and then converted to 2-chloro-5-chloromethylpyridine ([^{3}H]**3**) on refluxing in chloroform with thionyl chloride. [^{3}H]**3** was coupled to *N*-cyano-*N'*-methylacetamidine (**4**)[19] in refluxing acetonitrile in the presence of K_2CO_3 to give, after workup and preparative TLC first and then HPLC, [^{3}H]acetamiprid at a specific activity of 45 Ci/mmol, which is very close to the theoretical maximum tritium incorporation based on the percentage of ^{3}H in NaB^3H_4 (*i.e. ca.* 50% of the borohydride specific activity). The same procedure using NaB^2H_4 with 98% deuterium (Aldrich Chemical Co.) gave a compound with 90% deuterium incorporation. Tritium NMR (proton decoupled) showed that the major component contained two tritium atoms (singlet) and the minor compound only one tritium atom (singlet).



Scheme 1.

(a) NaB^3H_4, CH_3OH; or LiB^3H_4, THF; (b) $SOCl_2$, $CHCl_3$, reflux; (c) K_2CO_3, CH_3CN, reflux; (d) $H_2N(CH_2)_2NH_2$, CH_3CN, NaOH; (e) C_2H_5OH, reflux; (f) CNBr, $C_6H_5CH_3$.

In the synthesis of [^{3}H]CH-IMI and [^{3}H]desnitro-IMI, the chloronicotinoyl chloride (**1**) was reduced in tetrahydrofuran (THF) using freshly prepared LiB^3H_4 with a specific activity of 112 Ci/mmol to give 2-chloro-5-pyridylmethanol ([^{3}H]**2**). Conversion of [^{3}H]**2** to [^{3}H]**3** as above, and then reaction with ethylenediamine in acetonitrile and an aqueous solution of NaOH furnished [^{3}H]**5**. The diamine ([^{3}H]**5**) was dissolved in methanol and divided to two equal portions in separate flasks. To one of these portions 1,1-bis(methylthio)-2-nitroethylene (**6**) was added and the mixture was refluxed in ethanol. [^{3}H]CH-IMI with a

specific activity of 55 Ci/mmol (98% of theoretical maximal tritium incorporation, 50% of the borohydride specific activity) was isolated by HPLC purification[16] using methanol/water as the mobile phase on a LC-18 semi-preparative column to give 521 mCi.

[^{3}H]Desnitro-IMI was obtained by reacting a dry solution of [^{3}H]**5** in toluene with a solution of cyanogen bromide in toluene.[20,21] The product was isolated by preparative TLC in 30% radiochemical yield and has the same specific activity as [^{3}H]CH-IMI by comparing the ^{1}H NMRs of CH-IMI versus [^{3}H]CH-IMI, and those of desnitro-IMI versus [^{3}H]desnitro-IMI . Tritium NMR of both compounds showed only a singlet at the chemical shift of the methylene tritium resonance.

EXPERIMENTAL

General

HPLC was performed on a LC-18 DB or Vydac semi-preparative column (1.3 X 25 cm) with a mobile phase of methanol/water using Waters model 510 pumps. UV detection was at 270 nm for CH-IMI and 246 nm for acetamiprid on a Hewlett Packard 1040A diode array spectrophotometer. Radioactivity was monitored by an IN/US β-ram HPLC flow detector, using a lithium glass scintillant cell with an efficiency of ca. 0.5%. Liquid scintillation counting was performed with a Packard 1500 liquid scintillation system, using OptiFluor cocktail. Specific activities were determined by 1) comparison of the UV absorbance with that of a standard analytical sample and liquid scintillation counting of the isolated HPLC peak effluent or 2) using ^{1}H and ^{3}H NMR (IBM AF Spectrometer at 300 and 320 MHz, respectively, with $C^2H_3O^2H$ and a 5-mm probe). Preparative TLC was performed on precoated plates (silica gel GF, 20 X 20 cm, Analtech) with scraping of the gel inside sealed Atmosbags (Aldrich Chemical Co.) in a well-ventilated hood.

Syntheses of *N*-(2-chloro-5-pyridylmethyl)ethylenediamine (**5**), CH-IMI and desnitro-IMI

N-(2-Chloro-5-pyridylmethyl)ethylenediamine (**5**): To a mixture of ethylenediamine (1.86 g, 31.0 mmol) in acetonitrile (10.0 mL) and a 50% aqueous solution of NaOH (0.25 mL), stirred at 0 °C, was added 2-chloro-5-chloromethylpyridine (**3**) (1.0 g, 6.2 mmol) in acetonitrile (5.0 mL) dropwise. The mixture was then warmed to room temperature and stirred for 6 hr. Filtration and concentration *in vacuo* followed by preparative TLC purification (2.0 mm thickness) using 10% MeOH/$CHCl_3$ as eluent gave 1.7 g of **5** in 92% yield. ^{1}H NMR ($CDCl_3$) δ: 8.34(d, J=2.2 Hz, 1H, pyridyl), 7.68 (dd, J=2.2, 8.2 Hz, 1H, pyridyl), 7.28(d, J=8.2 Hz, 1H, pyridyl), 3.8(s, 2H, pyridyl-CH_2-), 2.84(t, J=6.4 Hz, 2H, -CH_2NH-), 2.71(t, J=6.4 Hz, 2H, -CH_2NH_2), 1.93(s, 3H, NH, NH_2). ^{13}C NMR ($CDCl_3$) δ: 150.1, 149.3, 138.7, 134.8, 123.8, 51.6 (pyridyl-CH_2-), 50.3 (-CH_2NH-), 41.5 (-CH_2NH_2).

CH-IMI: To a refluxing solution of 1,1-bis(methylthio)-2-nitroethylene (**6**) (5.5 g, 33.0 mmol) in absolute ethanol (30.0 mL) was added dropwise and with stirring the above compound (**5**) (5.75 g, 31 mmol) in ethanol (30.0 mL). The resulting solution was refluxed for 6 hr and then cooled to room temperature and concentrated *in vacuo*. The crude product was purified by preparative TLC (2.0 mm thickness) to give 6.5 g (82% yield). ^{1}H NMR ($CDCl_3$) δ: 8.72(s, NH, exchangeable in $C^2H_3O^2H$), 8.29(d, J=2.2 Hz, 1H), 7.56(dd, J=2.2, 8.2 Hz, 1H), 7.34(d, J=8.2 Hz, 1H), 6.66(s, 1H, =CH-NO2, exchangeable upon storing in $C^2H_3O^2H$ for >48 hr),[22] 4.33(s, 2H, pyridyl-CH_2-), 3.81(t, J=9.1 Hz, 2H, imidazolidinyl-CH_2-), 3.58(t, J=9.1 Hz, 2H, imidazolidinyl-CH_2-). ^{13}C NMR ($CDCl_3$) δ: 159.8 [=C(NH-)N], 151.7, 148.7, 137.9, 134.6, 124.8, 96.6 (=CH-NO_2), 48.5 (pyridyl-CH_2-), 46.5 (imidazolidinyl-CH_2-), 42.4 (imidazolidinyl-CH_2-). UV (absolute ethanol) λ_{max}=318, ϵ=11,000; λ_{max}=270, ϵ=4,788. FAB LR-MS: $C_{10}H_{11}ClN_4O_2H^+$ (255, 100%) for ^{35}Cl, (257, 33%) for ^{37}Cl. FAB HR-MS, MH^+: calculated 255.06488, found 255.06462.

Desnitro-IMI: To a solution of cyanogen bromide (0.5 g, 4.58 mmol) in toluene (15 mL) was added **5** (0.85 g, 4.58 mmol) in toluene (5.0 mL) dropwise at room temperature under nitrogen atmosphere. After the mixture was stirred for 3 hr, the precipitate was filtered and washed with toluene to give 1.18 g of a yellowish powder in 89% yield. ^{1}H NMR ($CDCl_3$) δ: 8.30(d, J=2.1 Hz, 1H), 7.64(dd, J=2.2, 8.3 Hz, 1H), 7.32(d, J=8.3 Hz, 1H), 4.87(s, NH, exchangeable in $C^2H_3O^2H$), 4.36(s, 2H, pyridyl-CH_2-), 3.38(m, 4H, imidazolidinyl-CH_2CH_2-), 1.82(s, 1H, NH, exchangeable in $C^2H_3O^2H$). ^{13}C NMR ($CDCl_3$) δ: 162.6 (HN=C), 150.5, 148.9, 138.6, 131.7, 124.2, 44.5 (pyridyl-CH_2-), 44.4 (imidazolidinyl-CH_2-), 37.9 (imidazolidinyl-CH_2-). UV (absolute ethanol) λ_{max}=268, ϵ=3,889. FAB LR-MS: $C_9H_{11}ClN_4H^+$ (211, 100%) for ^{35}Cl, (213, 33%) for ^{37}Cl. FAB HR-MS, MH^+: calculated 211.07505, found 211.07497.

Synthesis of acetamiprid and [^{3}H]acetamiprid

Acetamiprid was synthesized as described earlier.[19]

[^{3}H]Acetamiprid: To a solution of freshly-prepared NaB^3H_4 (380 μl, 3.8 Ci, 90 Ci/mmol, 0.042 mmol, NaOH/MeOH, 0.1 N) in methanol (2.0 mL) was added 6-chloronicotinoyl chloride (**1**)(40 mg, 0.22 mmol). The resulting cloudy mixture was stirred at room temperature for 2 hr, after which it was concentrated to 0.5 mL and aqueous NaH_2PO_4 (1.0 M, 1.0 mL) was added. The aqueous phase was extracted with CH_2Cl_2, dried ($MgSO_4$), and filtered through glass wool packed inside a pasteur pipet. The CH_2Cl_2 solution was evaporated using a stream of nitrogen and the oily residue was dissolved in $CHCl_3$ (5.0 mL) containing thionyl chloride (1.5 mL). The resulting solution was refluxed for 2 hr and then cooled

to room temperature and lyophilized. The residue was dissolved in CH_3CN (2.0 mL) and counted (1.15 Ci). To this solution of [^{3}H]**3** (0.5 Ci) in CH_3CN (4.0 mL) was added K_2CO_3 (150 mg) and **4** (40 mg, 0.41 mmol). The mixture was refluxed overnight. After cooling to room temperature, water was added and the aqueous phase was extracted with CH_2Cl_2, dried ($MgSO_4$) and filtered. The solvent was evaporated using a stream of nitrogen and the residue was dissolved in methanol (1.0 mL) and counted to give 335 mCi. Preparative TLC (0.5 mm thickness) using 10% MeOH/$CHCl_3$ as eluent, scraping the layer corresponding to the product and extracting with MeOH gave 160 mCi of crude product. A second purification by HPLC on a reverse phase column gave 40 mCi of > 98% pure [^{3}H]acetamiprid, R_t = 7.8 min with a flow of 1.5 mL/min (35% MeOH/H_2O). Specific activity = 45 Ci/mmol (based on HPLC). ^{3}H NMR ($C^2H_3O^2H$) δ: (^{1}H-decoupled) 4.74(s, ^{3}H-^{3}H), 4.77(s, ^{1}H-^{3}H). ^{1}H NMR ($C^2H_3O^2H$) δ: 8.34(d, J=2.2 Hz, 1H), 7.73(dd, J=2.2, 8.2 Hz, 1H), 7.45(d, J=8.2 Hz, 1H), 4.78 (a small peak resulting from CH_2), 4.72 (a smaller peak from CH-^{3}H), 3.16(s, N-CH_3), 2.46(s, CH_3).

Synthesis of [^{3}H]**5**, [^{3}H]CH-IMI and [^{3}H]desnitro-IMI

Synthesis of [^{3}H]**5**: To a solution of 6-chloronicotinoyl chloride (**1**) (20 mg, 0.12 mmol) in dry THF (0.4 mL), stirred at 0°C, was added a suspension of freshly prepared LiB^3H_4 in THF (0.3 mL, 3.0 Ci, 112 Ci/mmol, 0.27 mmol). The mixture was then warmed to room temperature and stirred under nitrogen atmosphere for 1.5 hr. Ethyl acetate (0.3 mL) was added followed by a 1 M solution of aqueous NaH_2PO_4 (0.3 mL) and the resulting mixture was stirred vigorously for 10 min. After allowing the solution to settle, the organic phase was pipeted out and evaporated under a stream of nitrogen. $CHCl_3$ (5.0 mL) and thionyl chloride (0.5 mL) were added and the solution was refluxed for 2 hr. Most of the solvent and thionyl chloride were evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ and a saturated solution of $NaHCO_3$ was added. The aqueous phase was extracted with $CHCl_3$, and the organic extract was run through a short column of $MgSO_4$ and concentrated using a stream of nitrogen. Acetonitrile (3.0 mL) and ethylenediamine (0.1 mL) were added followed by a solution of 50% aqueous NaOH (0.05 mL) and stirring at room temperature for 2 hr. The reaction was then filtered and lyophilized overnight to give 2.6 Ci of the ethylenediamine derivative ([^{3}H]**5**).

[^{3}H]CH-IMI: Half of the [^{3}H]**5** (1.3 Ci) in absolute ethanol (3.0 mL) was added to 1,1-bis(methylthio)-2-nitroethylene (**6**) (10 mg, 0.060 mmol) and the mixture was refluxed for 3 hr. It was then cooled to room temperature and lyophilized overnight. The crude product (1.23 Ci) was purified by HPLC using a LC-18 DB column and 50% MeOH/H_2O at a flow of 3.0 mL/min (R_t=5.34 min) or on

the Vydac LC-18 column and 40% MeOH/H_2O at a flow of 3.0 mL/min (R_t=7.02 min) to give 522 mCi in 33% radiochemical yield (specific activity = 55 Ci/mmol, based on NMR). ^{3}H NMR ($C^2H_3O^2H$) (^{1}H-decoupled) δ: 4.71(s, ^{3}H-^{3}H). ^{1}H NMR ($C^2H_3O^2H$) δ: 8.34(d, J=2.1 Hz, 1H), 7.80(dd, J=2.1, 8.2 Hz, 1H), 7.48(d, J=8.2 Hz, 1H), 3.68(m, 4H).

[^{3}H]Desnitro-IMI: The other half of the ethylenediamine derivative ([^{3}H]5) (1.3 Ci) was lyophilized to dryness and then dissolved in dry toluene (3.0 mL). A solution of cyanogen bromide (7.0 mg, 0.066 mmol) in toluene (1.0 mL) was added dropwise at room temperature and the mixture was stirred for 6 hr. The reaction was lyophilized overnight to give 1.4 Ci of the crude material which was purified by preparative TLC (1.0 mm thickness) using $CHCl_3$/MeOH (70:30) as eluent. The product was isolated as before and concentrated to give 457 mCi (specific activity = 55 Ci/mmol based on NMR). ^{3}H NMR ($C^2H_3O^2H$) (^{1}H-decoupled) δ: 4.5(s, ^{3}H-^{3}H). ^{1}H NMR ($C^2H_3O^2H$) δ: 8.36(d, J=2.1 Hz, 1H), 7.82(dd, J=2.1, 8.2 Hz, 1H), 7.46(d, J=8.2 Hz, 1H), 3.64(m, 4H).

ACKNOWLEDGMENT

The project described was supported by Grant No. P01 ES00049 from the National Institute of Environmental Health Sciences, NIH to the Environmental Chemistry and Toxicology Laboratory. The National Tritium Labelling Facility staff were supported by the Biomedical Research Technology Program, National Center for Research Resources, NIH, under Grant P41 RR01237, and through Contract DE-AC03-76SF00098 with the Department of Energy. We thank our laboratory colleague Gary Quistad for useful information and discussions.

REFERENCES

1. Tomizawa, M. and Yamamoto, I. -J. Pesticide Sci. 18:91 (1993).
2. Yamamoto, I., Yabuta, G., Tomizawa, M., Saito, T., Miyamoto, T., and Kagabu, S. -J. Pesticide Sci. 20:33 (1995).
3. Feuer, H. and Lawrence, J. P. -J. Org. Chem. 37:3662 (1972).
4. Soloway, S. B., Henry, A. C., Kollmeyer, W. D., Padgett, W. M., Powell, J. E., Roman, S. A., Tieman, C. H., Corey, R. A., and Horne, C. A. -In Pesticide and Venom Neurotoxicity (Shankland, D. L., Hollingworth, R. M., and Smyth Jr., T., Eds.), Plenum, New York, pp. 153-158 (1978).
5. Kagabu, S., and Medej, S. -Biosci. Biotech. Biochem. 59:980 (1995).
6. Kleir, D., Holden, I., Casida, J. E., and Ruzo, L. O. -J. Agric. Food Chem. 33:998 (1985).
7. Kagabu, S., Moriya, K., Shibuya, K., Hattori, Y., Tsuboi, S., and Shiokawa, K. -Biosci. Biotech. Biochem. 56:362 (1992).

8. Shiokawa, K., Tsuboi, S., Iwaya, K., and Moriya, K. -J. Pesticide Sci. 19:329 (1994).

9. Liu, M. -Y., Lanford, J., and Casida, J. E. -Pestic. Biochem. Physiol. 46:200 (1993).

10. Klein, O. Poster presented in July at 8th Internat. Congr. Pestic. Chem., Washington, D.C. (1994).

11. Takahashi, H., Mitsui, J., Takakusa, N., Matsuda, M., Yoneda, H., Suzuki, J., Ishimitsu, K., and Kishimoto, T. -Brighton Crop Prot. Conf. Pests. Dis. 2:89 (1992).

12. Leicht, W. -Pestic. Outlook 4:17 (1993).

13. Chao, S. L., Dennehy, T. J., and Casida, J. E. -Pestic. Biochem. Physiol., submitted (1996).

14. Latli, B., and Casida, J. E. -J. Labelled Compd. Radiopharm. 31:609 (1992).

15. Liu, M.-Y., and Casida, J. E. -Pestic. Biochem. Physiol. 46:40 (1993).

16. Liu, M.-Y., Latli, B., and Casida, J. E. -Pestic. Biochem. Physiol. 50:171 (1994).

17. Andres, H., Morimoto, H., and Williams, P. G. -J. Chem. Soc., Chem. Commun. 627 (1990).

18. Than, C., Morimoto, H., Andres, H., and Williams, P. G. -J. Labelled Compd. Radiopharm., in press (1996).

19. Liu, M. -Y., Latli, B., and Casida, J. E. -Pestic. Biochem. Physiol. 52:170 (1995).

20. Ishikawa, F., Kosasayama, A., Nakamura, S., and Konno, T. -Chem. Pharm. Bull. 26:3658 (1978).

21. Shiokawa, K., Tsuboi, S., Kagabu, S., and Moriya, K., -European Patent EP 0 192 060 A1 (1986).

22. Rajappa, S. -Tetrahedron 37:1453 (1981).