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# SPECIFICALLY DEUTERATED AND TRITIATED AUXINS

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**Key Word Index**—Auxin; azido auxin; indole-3-acetic acid; 5-azidoindole-3-acetic acid; naphthalene-1-acetic acid; radiolabeling; photoaffinity labeling; synthesis.

**Abstract**—Regiospecific syntheses of monodeuterated and monotritiated natural auxin (indole-3-acetic acid), a synthetic auxin (naphthalene-1-acetic acid) and a photoaffinity labeling auxin (5-azidoindole-3-acetic acid) are described. These syntheses provide benzene-ring tritiated auxins for use in reversible and covalent binding studies.

## INTRODUCTION

[$^{14}$C]IAA has been used extensively to study the action and metabolism of auxin in plants [1, 2], while [$^{14}$C]NAA has been featured in recent efforts to demonstrate auxin binding and to isolate auxin receptors [3, 4]. Both [$^{14}$C]IAA and [$^{14}$C]NAA are commercially available, and their syntheses have been described [5–9]. Compared with $^{3}$H, however, $^{14}$C suffers the inherent disadvantage of low maximum theoretical activity [10, 11], which limits the scope of $^{14}$C-labeled compounds in auxin binding studies. Despite its obvious potentiality, [$^{3}$H]NAA has not been used for this purpose, and the only reported synthesis of the compound is Evans' preparation of low specific activity, generally labeled NAA [12]. Although [5-$^{3}$H]IAA (**2c**) has been used occasionally for auxin binding studies [13–16], its method of preparation apparently remains proprietary information and, until recently, it was though to be an especially unstable form of IAA [17–19]. The finding by Pengelley [20] that [5-$^{3}$H]IAA (**2c**) is no more unstable than [carboxy-$^{14}$C]IAA suggests that [$^{3}$H]IAA may indeed by a useful, sensitive probe for auxin binding under conditions in which oxidation is controlled or prevented.

Here, we present a synthesis of [5-$^{3}$H]IAA (**2c**) from 5-BrIAA (**1**), which can be adapted to the preparation of [$^{2}$H]- or [$^{3}$H]IAA labeled in any ring position, given the appropriate BrIAA. We also describe the preparation of [4-$^{3}$H]NAA (**16c**), which, like NAA (**16a**) itself, has greater chemical stability than IAA (**2**) and which is useful, therefore, when oxidation of [5-$^{3}$H]IAA (**2c**) by air or by enzymes creates problems. Finally, in a continuation of our study of azido auxins as photoaffinity labeling agents for the isolation of auxin receptors [21], we describe the synthesis of 5-$N_3$-[7-$^{3}$H]IAA (**14c**). All three tritiated auxins are labeled in the benzene ring and thus preclude loss of $^{3}$H through facile metabolic or chemical exchange [22, 23]. Both [5-$^{3}$H]IAA (**2c**) and 5-$N_3$-[7-$^{3}$H]IAA (**14c**) have the further advantage over [$^{3}$H]NAA of requiring no assumptions about the identity or non-identity of NAA and IAA receptors or binding sites.

## RESULTS AND DISCUSSION

### *Synthesis of* 5-$N_3$*IAA* (**14a**), 5-$N_3$-[7-$^{2}$H]*IAA* (**14b**) *and* 5-$N_3$-[7-$^{3}$H]*IAA* (**14c**)

IAA (**2a**) was prepared from 5-BrIAA (**1**) by hydrogenolysis over Raney nickel. Attempts to obtain [5-$^{2}$H]IAA by this procedure (Scheme 1), substituting $^{2}H_2$ for $^{1}H_2$, gave only 5% deuterium incorporation, due to extensive dilution by $^{1}H_2$ adsorbed on the catalyst. Deuterolysis over palladium on charcoal, however, gave a high degree of deuterium incorporation (75% monodeuteration by isotope ratio mass spectral analysis and *ca* 79% by integration of the 360 MHz $^{1}$H NMR spectrum). Comparison of the $^{1}$H NMR spectrum of this sample with a fully coupled spectrum of authentic IAA and with a decoupled spectrum of authentic IAA (**2a**) irradiated at H–5 established that deuteration had occurred almost

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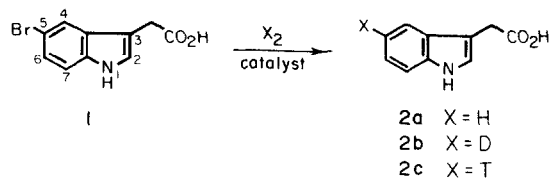
Abbreviations: 5-BrIAA, 5-bromoindole-3-acetic acid; 4-BrNAA, 4-bromonaphthalene-1-acetic acid; 7-Br-5$NO_2$IAA, 7-bromo-5-nitroindole-3-acetic acid; [carboxy-$^{14}$C]IAA, indole-3-[carboxy-$^{14}$C]acetic acid; DDQ, 2,3-dichloro-5, 6-dicyanobenzoquinone; EIMS, electron impact mass spectrometry; FDMS, field desorption mass spectrometry; [5-$^{2}$H]IAA, [5-$^{2}$H]indole-3-acetic acid; [5-$^{3}$H]IAA, [5-$^{3}$H]indole-3-acetic acid; [4-$^{2}$H]NAA, [4-$^{2}$H]naphthalene-1-acetic acid; [4-$^{3}$H]NAA, [4-$^{3}$H]naphthalene-1-acetic acid; IAA, indole-3-acetic acid; NAA, naphthalene-1-acetic acid; 5-$NH_2$IAA, 5-aminoindole-3-acetic acid; 5-$NH_2$-[7-$^{2}$H]IAA, 5-amino-[7-$^{2}$H]indole-3-acetic acid; 5-$NH_2$-[7-$^{3}$H]IAA, 5-amino-[7-$^{3}$H]indole-3-acetic acid; 5-$N_3$IAA, 5-azidoindole-3-acetic acid; 5-$N_3$-[7-$^{2}$H]IAA, 5-azido-[7-$^{2}$H]indole-3-acetic acid; 5-$N_3$-[7-$^{3}$H]IAA, 5-azido-[7-$^{3}$H]indole-3-acetic acid; 5-$NO_2$IAA, 5-nitroindole-3-acetic acid; TSP, sodium 3-(trimethylsilyl) tetradeuteropropionate.




Scheme 1.

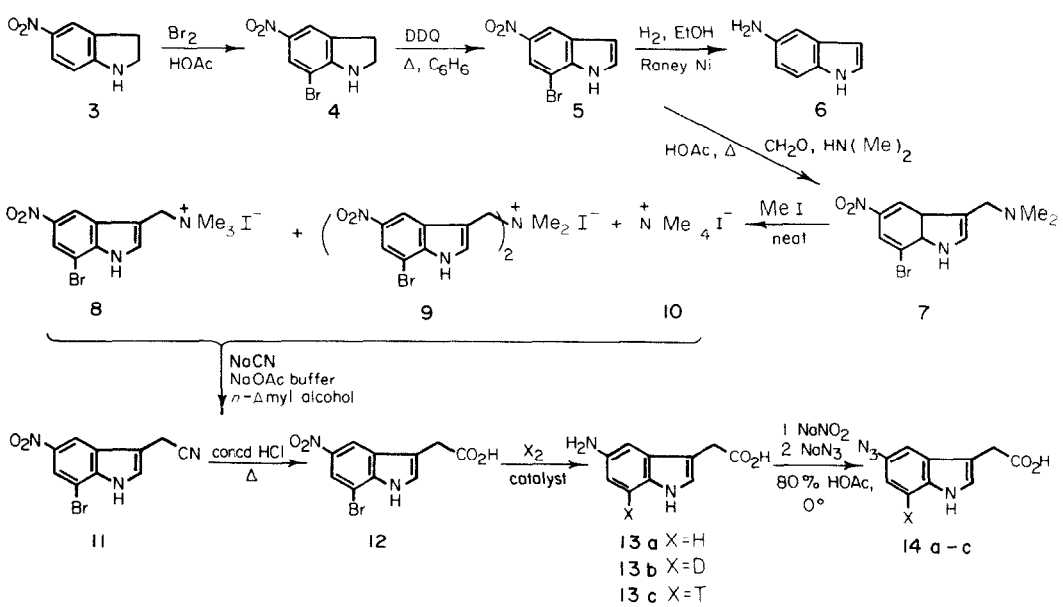
exclusively at the 5-position. Tritiolysis of 5-BrIAA (**1**) over palladium on charcoal gave [$^3$H]IAA (**2c**) that was assumed to be labeled at the 5-position by analogy with the results for the deuterated compound **2b** and in agreement with previous reports of the tritiolysis of 5-bromotryptophan under similar conditions to give [5-$^3$H]tryptophan [24, 25]. Both high (16.6 Ci/mmol) and low (34.9 mCi/mmol) specific activity [5-$^3$H]IAA were stable for at least 1 month when stored in the dark in solution at $-20°$, absolute ethanol being preferable to diethyl ether as solvent.

5-$N_3$IAA (**14a**) was prepared as shown in Scheme 2, beginning with the bromination of 5-nitroindoline (**3**) to form 7-bromo-5-nitroindoline (**4**) [26]. Oxidation of the indoline **4** to the corresponding indole **5** with chloranil [27] failed, but the stronger oxidizing agent DDQ gave the desired 7-bromo-5-nitroindole (**5**) in good yield. Treatment of **5** with hydrogen over Raney nickel effected both reduction of the nitro group and hydrogenolysis of the halide to give 5-aminoindole (**6**), indicating that an analogous reaction would be feasible after elaboration of the side chain. Introduction of the side chain by a Mannich reaction [21] worked well, despite the presence of two electron-withdrawing groups in the benzene ring, yielding 7-bromo-5-nitrogramine (**7**). Methylation of **7** in neat methyl iodide gave a 4:1 mixture of 7-bromo-5-nitrogramine methiodide (**8**) and 3, 3′-bis(7-bromo-5-nitroindolylmethyl)dimethylammonium iodide (**9**), in agreement with results obtained earlier for alkylation of gramine and other nitro-substituted gramines [28]. Formation of 7-bromo-5-nitroindole-3-acetonitrile (**11**) from the crude methiodide mixture (**8–10**) was accomplished by displacement with cyanide, and the resulting nitrile **11** was hydrolysed to 7-Br-5-$NO_2$IAA (**12**). by heating in concentrated hydrochloric acid.

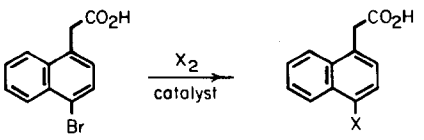
Reduction of the acid **12** was achieved under several conditions. In general, procedures employing palladium on charcoal were superior to those using Raney nickel. Conversion of the resulting 5-$NH_2$IAA (**13a**) to 5-$N_3$IAA (**14a**) by diazotization and displacement with $N_3^-$ has been described [21]. 5-$N_3$-[7-$^2$H]IAA (**14b**) was prepared by deuteration of 7-Br-5-$NO_2$IAA (**12**) over palladium on charcoal in aqueous potassium hydroxide. After the crude product was converted to 5-$N_3$-[7-$^2$H]IAA (**14b**), mass spectrometry and integration of the 360 MHz $^1$H NMR spectrum demonstrated 75% monodeuteration and *ca* 68% deuteration, respectively. Comparison of the $^1$H NMR spectrum of this sample with a fully coupled spectrum of authentic 5-$N_3$IAA [21] and a decoupled spectrum of authentic 5-$N_3$IAA [21] irradiated at H–7 clearly showed that deuteration had occurred predominantly at the 7-position. Tritiation of 7-Br-5-$NO_2$IAA (**12**) and conversion of the amine (**13c**) to 5-$N_3$-[7-$^3$H]IAA (**14c**) were performed in a similar manner, substituting $^3H_2$ for $^2H_2$. By analogy with the results for [5-$^2$H]IAA (**2b**) and 5-$N_3$-[7-$^2$H]IAA (**14b**), the label was assumed to be in the 7-position. 5-$N_3$-[7-$^3$H]IAA (**14c**) (111 mCi/mmol) was stable for at least 1 month when stored in benzene–ethyl acetate (2:1) at $-20°$.

### *NAA* (**16a**), [4-$^2$H]*NAA* (**16b**) *and* [4-$^3$H]*NAA* (**16c**)

NAA (**16a**), [4-$^2$H]NAA (**16b**) and [4-$^3$H]NAA (**16c**) were prepared by hydrogenolysis, deuterolysis and tritiolysis of 4-BrNAA (**15**) over palladium on char-




Scheme 2.




15 16a X = H
16b X = D
16c X = T

Scheme 3.

coal, as shown in Scheme 3. As with the indoles, hydrogenolysis demonstrated the facile replacement of the bromo group, while deuterolysis showed that a high degree of specific labeling could be achieved, as judged by $^1$H NMR spectroscopic and mass spectral evidence. Although the 360 MHz $^1$H NMR spectrum of NAA (**16a**) is considerably more complex than that of IAA (**2a**) or 5-$N_3$IAA (**14a**), the spectrum can be interpreted by assuming the manifestation of several effects characteristic of naphthalene systems [29]: (a) $\alpha$-hydrogens resonate downfield from $\beta$-hydrogens; (b) hydrogens on the ring bearing electron-donating substituents are shielded relative to analogous ($\alpha$ or $\beta$) hydrogens in the other ring; and (c) the $\alpha$-hydrogen peri to a substituent resonates downfield from other $\alpha$-hydrogens. The fully coupled spectrum of NAA (**16a**) and the results of homonuclear decoupling experiments on NAA (**16a**) irradiated sequentially at each ring hydrogen are consistent with assignments based on these assumptions. Comparison of the 360 MHz $^1$H NMR spectrum of [4-$^2$H]NAA (**16b**) with the fully coupled spectrum of an authentic sample of NAA and with a decoupled spectrum of authentic NAA irradiated at H-4 establish that deuteration occurred almost exclusively at the 4-position. Integration of the $^1$H NMR spectrum of [4-$^2$H]NAA (**16b**) showed *ca* 33% residual $^1$H at the 4-position, in agreement with the mass spectrum isotope ratio analysis, which revealed 70% monodeuteration. On the basis of the results for deuterolysis, tritiolysis was assumed to yield [4-$^3$H]NAA (**16c**). The product (**16c**) showed no detectable decomposition after storage in absolute ethanol at $-20°$ for 1 month.

## *Conclusion*

We have described the preparation of specifically deuterated and tritiated IAA, 5-$N_3$IAA and NAA from brominated precursors. For both the indoles and the naphthalene derivatives, hydrogenolysis established the feasibility of tritiolysis of the bromo group, while deuterolysis established the position and realizable extent of isotopic substitution by mass spectral and $^1$H NMR spectroscopic analysis. In each case, tritiolysis gave stable products of high radiochemical purity.

## EXPERIMENTAL

Mps were determined on a Thomas-Hoover Unit-Melt capillary apparatus and are corr. When 90 MHz $^1$H NMR spectra were obtained in DMSO-$d_6$, TMS was used as an int. standard, but when 360 MHz spectra were obtained in this solvent, residual $^1$H in DMSO-$d_6$ served as an int. standard. For $^1$H NMR spectra obtained in $D_2O$–NaOD, TSP was the int. standard. MS were obtained by J. Carter Cook and his staff at the University of Illinois. EIMS were obtained on a Varian-MAT CH-5 spectrometer coupled with a 620i computer and STATOS recorder. EIMS isotope ratio measurements, which represent the average of five consecutively recorded partial spectra, were made on the same instrument at 70 eV, using an oscillographic recorder. FDMS were obtained on a Varian-MAT 731 spectrometer equipped with a Varian-MAT combination electron impact–field desorption source. FDMS isotope ratios were obtained on the same instrument with a multichannel analyser, scanning *m/z* 216–218 at 0 mA. Microanalyses were performed by Mr. Josef Nemeth and his staff at the University of Illinois. Analytical TLC was performed on Brinkmann Si gel plates with fluorescent indicator, using EtOAc–*iso*-PrOH–conc. $NH_4OH$ (45:35:20) (solvent A), EtOAc–*iso*-PrOH–$H_2O$ (65:25:10) (solvent B), or $H_2O$–HOAc–$CCl_4$ (3:5:10) (solvent C, both phases) as eluant.

Prep. TLC was conducted on 20 × 20 cm Brinkmann Si gel pates, 2.0 mm thick, with fluorescent indicator. Unless otherwise noted, visualization was under 254 and 365 nm light. For radiochromatography, a Packard radiochromatogram scanner was also used for detection. EtOAc was dried and distilled according to published directions [30]. 5-BrIAA (**1**), 5-nitroindoline (**3**) and 5-aminoindole · HCl (**6** · HCl) were purchased from Aldrich. Authentic samples of NAA (**16a**) and IAA (**2a**), purchased from Eastman, were recrystallized ×3 from $H_2O$. The purity of 5-BrIAA (**1**), 5-nitroindoline (**3**), NAA (**16a**) and IAA (**2a**) was verified by mp, TLC, and 360 MHz $^1$H NMR. The preparation of authentic samples of 5-$NH_2$IAA (**13a**) and 5-$N_3$IAA (**14a**) by different route than the one outlined in Scheme 3 has been described [21]. Raney nickel (Mo-activated, No. 30) was obtained from W. R. Grace; Pd on charcoal was from Englehard. $^2H_2$ (research grade) was bought from Air Products and Chemicals; $^3H_2$ (carrier free) came from New England Nuclear.

*Indole-3-acetic acid* (**2a**). In a 500 ml hydrogenation bottle, 0.150 g 5-BrIAA (**1**) was dissolved in 50 ml absolute EtOH. After the bottle was flushed with $N_2$, Raney nickel and 1 equivalent NaOAc · $3H_2O$ were added. The reaction mixture was shaken under $H_2$ at 45 psi on a Parr apparatus at room temp. for 4 hr. The catalyst was removed by filtration and washed ×3 with 15 ml portions of absolute EtOH. The filtrate and combined washings were evaporated to dryness at 30° under red. pres., giving a white ppt, which was collected by filtration after addition of 2 ml $H_2O$ and washed twice with 1 ml $H_2O$. Drying overnight at 56° under vacuum gave IAA (**2a**) as white crystals weighing 0.054 g (52%): mp 165–166° (lit. [31] 168–170°). TLC, solvent A, $R_f = 0.28$. Similar results were obtained when dry EtOAc was used as solvent. $^1$H NMR (360 MHz, DMSO-$d_6$): $\delta$3.64 (2H, *s*, $CH_2$), 6.98 [1H pseudo *t* (overlapping *dd*), H-5, $J_{5,4} = 7.9$ Hz, $J_{5,6} = 7.2$ Hz], 7.07 [1H, pseudo *t* (overlapping *dd*), H-6, $\cdot J_{6,5} = 7.2$ Hz, $J_{6,7} = 8.1$ Hz], 7.23 (1H, *d*, H-2, $J_{2,NH} = 2.0$ Hz), 7.35 (1H, *d*, H-7, $J_{7,6} = 8.1$ Hz), 7.49 (1H, *d*, H-4, $J_{4,5} = 7.9$ Hz), irradiation at 6.98 caused collapse of *d* at $\delta$ 7.49 to *s*.

[5-$^2$H]*Indole-3-acetic acid* (**2b**). *Method A.* 5-BrIAA (0.168 g, **1**) was placed in a 50 ml round-bottomed flask in 15 ml dry EtOAc. Raney nickel, washed ×3 with dry EtOAc, and 0.055 g NaOAc were added. The flask was attached to a vacuum line, the reaction mixture was frozen, and the line was evacuated. Sufficient $^2H_2$ was introduced into the apparatus to bring the system to atmospheric pres., and the

reaction mixture was stirred overnight. The mixture was removed from the line, filtered through a medium-frit sintered-glass funnel, and the solvent was removed under red. pres. at room temp. Aq. NaOH (2 ml 2 N) was added, and the soln was evaporated to dryness under red. pres. at room temp. Following addition of $H_2O$ and evaporation as before, $\times 4$, the residue was dissolved in 15 ml $H_2O$, acidified to pH 2.8 with 1N HCl and extracted $\times 4$ with 25 ml portions $Et_2O$. After the $Et_2O$ extracts were evaporated to dryness under red. pres. at room temp., the residue was purified by prep. TLC (solvent A, $R_f = 0.27$, eluted from Si gel with EtOAc–absolute EtOH, 1:9) giving 0.047 g (40%) [5-$^2$H]IAA (**2b**) as white crystals: mp 167–169.5°. $^1$H NMR (360 MHz, DMSO-$d_6$) was indistinguishable from that of IAA (**2a**). EIMS isotope ratio analysis showed 5% monodeuterated product (**2b**), the remainder being undeuterated IAA (**2a**). *Method B.* In a 25 ml round-bottomed flask was placed 0.150 g 5-BrIAA (**1**), 10 ml $H_2O$ containing 0.075 g KOH, 0.100 g 5% Pd on charcoal, and a small magnetic follower. The flask was attached to a vacuum line, the mixture frozen, and the line evacuated. $^2H_2$ was introduced into the line to atmospheric pres. and the reaction mixture was stirred for 2 hr at room temp., at which time analytical TLC (solvent A) showed that no starting material was present. The reaction vessel was removed from the vacuum line, and the contents were filtered through a medium-frit sintered-glass funnel. The pH of the filtrate was adjusted to 11 with 1N KOH and the soln was washed twice with 25 ml $Et_2O$. After the aq. phase was acidified to pH 3.0, it was extracted $\times 4$ with 25 ml portions $Et_2O$. Evaporation of the $Et_2O$ extracts under red. pres. at room temp. gave 0.085 g [5-$^2$H]IAA (**2b**) as pink crystals (85%): mp 167–169°. TLC, solvent A, $R_f = 0.27$. $^1$H NMR (360 MHz, DMSO-$d_6$): $\delta$3.64 (2H, *s*, $CH_2$), 6.97 [0.21 H pseudo *t* (overlapping *dd*), residual H-5], 7.06 (1H, *d*, H-6, $J_{6,7} = 8.2$ Hz), 7.21 (1H, *br s*, H-2), 7.33 (1H, *d*, H-7, $J_{7,6} = 7.6$ Hz), 7.48 (1H, *br s*, H-4). EIMS isotope ratio analysis showed 20% undeuterated, 75% monodeuterated and 4% dideuterated product.

[5-$^3$H]*Indole-3-acetic acid* (**2c**). [5-$^3$H]IAA (**2c**) was prepared by tritiolysis of 5-BrIAA(**1**) according to Method B above for [5-$^2$H]IAA (**2b**). From 0.026 g 5-BrIAA (**1**) and a mixture of $^3H_2$ and $^1H_2$, was obtained 0.017 g (95%) [5-$^3$H]IAA (**2c**) containing 3.39 mCi, sp. act. 34.9 mCi/mmol. TLC, solvent A, $R_f = 0.29$. The product was dissolved in 20 ml $Et_2O$ and stored in the dark at −20°. After 1 month, radiochromatography (solvent A) showed the product to be more than 99% pure. This procedure was repeated on 0.022 g 5-BrIAA (**1**) using a 2:7 mixture of $^3H_2$–$^1H_2$ to give 0.0145 g (96%) [5-$^3$H]IAA (**2c**) containing 1374 mCi, sp. act. 16.6 Ci/mmol. TLC, solvent A, $R_f = 0.34$. Radiochromatography (solvent A) showed the product (**2c**) to be 98% radiochemically pure. Storage of high sp. act. [5-$^3$H]IAA (**2c**) in $Et_2O$ at −20° in the dark for 2 weeks led to 20% decomposition according to radiochromatography, whereas, after storage in the dark in absolute EtOH at −20° for 1 month, no decomposition was detectable by radiochromatography.

*7-Bromo-5-nitroindoline* (**4**). The procedure of Gall *et al.* [26] was used to brominate 15.2 g 5-nitroindoline (**3**) to give, after recrystallization from EtOH–$H_2O$, 20.5 g 7-bromo-5-nitroindoline (**4**) as orange crystals (91%): mp 153–154° (lit. [26] 149–151°). TLC, $CHCl_3$, $R_f = 0.36$. $^1$H NMR (90 MHz, DMSO-$d_6$): $\delta$3.46 (2H, *t*, C-3 $CH_2$, $J_{3,2} = 8.1$ Hz), 4.00 (2H, *t*, C-2 $CH_2$, $J_{2,3} = 8.1$ Hz), 7.52 (1H, *br s*, NH, exchanges with $D_2O$), 8.10 (1H, *br m*, H-4, collapses to *d* with $D_2O$, $J_{4,6} = 2.2$ Hz), 8.33 (1H, *d*, H-6, $J_{6,4} = 2.2$ Hz).

*7-Bromo-5-nitroindole* (**5**). A 250 ml round-bottomed flask was charged with 2.70 g 7-bromo-5-nitroindoline (**4**) and 2.52 g DDQ (1 equivalent) in 100 ml $C_6H_6$. The reaction mixture was heated at reflux for 6 hr and then allowed to cool to room temp. The resulting yellow-orange ppt was collected by filtration and washed $\times 3$ with $C_6H_6$. The combined filtrate and washings were saved, and the solid was extracted in a Soxhlet apparatus with 100 ml $C_6H_6$ for 48 hr. The $C_6H_6$ from extraction and filtration were combined and evaporated to dryness at room temp. under red. pres. Recrystallization of the resulting yellow-green powder from EtOH–$H_2O$ gave 2.34 g 7-bromo-5-nitroindole (**5**) as yellow crystals (91%): mp 201–203°. TLC, solvent B, $R_f = 0.80$. $^1$H NMR (90 MHz, DMSO-$d_6$): $\delta$6.90 (1H, *dd*, H-3, $J_{3,2} = 3.0$ Hz, $J_{3,NH} = 2.0$ Hz, collapses to *d* with $D_2O$, $J_{3,2} = 3.0$ Hz), 7.67 [1H, pseudo *t* (overlapping *dd*), H-2, $J_{2,3} = J_{2,NH} = 3.0$ Hz, collapses to *d* with $D_2O$, $J_{2,3} = 3.0$ Hz], 8.18 (1H, *d*, H-4, $J_{4,6} = 2.2$ Hz), 8.62 (1H, *d*, H-6, $J_{6,4} = 2.2$ Hz), 12.09 (1H, *br s*, NH exchanges with $D_2O$). EIMS 10 eV, *m/z* (rel. int.): 242, 240 $[M]^+$ (83, 100), 196, 194 $[M - NO_2]^+$ (27, 23), 115 $[M - NO_2 - Br]^+$ (27). Found: C, 40.02; H, 2.00; N, 11.56; Br, 33.19. $C_8H_5N_2O_2Br$ requires: C, 39.86; H, 2.09; N, 11.62; Br, 33.15%.

*5-Aminoindole* (**6**). In a 500 ml hydrogenation bottle, 0.150 g 7-bromo-5-nitroindole (**5**) was dissolved in 100 ml absolute EtOH. The bottle was flushed with $N_2$, and 0.085 g NaOAc · $3H_2O$ (1 equivalent) and Raney nickel were added. The reaction mixture was shaken for 1 hr at room temp. on a Parr apparatus under 45 psi $H_2$. The catalyst was removed by filtration and washed $\times 3$ with 10 ml portions of absolute EtOH. The combined filtrate and washings were evaporated to dryness at 30° under red. pres., giving a pink solid, which was triturated with hot $C_6H_6$. After insoluble material had been removed by filtration, the $C_6H_6$ was evaporated to dryness at 30° under red. pres., giving a white solid, which was dissolved in $Et_2O$. Addition of petrol produced a white ppt, which was collected by filtration. Air drying gave 5-aminoindole (**6**) as 0.048 g grey crystals (54%): mp 126–129° (lit. [32] 125–127°). TLC, solvent B, visualization by $I_2$, $R_f = 0.65$, which was identical to the $R_f$ for an authentic sample of 5-aminoindole hydrochloride (**6** · HCl).

*7-Bromo-5-nitrogramine* (**7**). In a 50 ml round-bottomed flask was placed 14.0 g 7-bromo-5-nitroindole (**5**) and 20 ml glacial HOAc. To this was added with stirring a soln containing 10 ml glacial HOAc, 4.7 g aq. 37% $CH_2O$ and 6.5 g 40% aq. $NHMe_2$. The flask was fitted with a reflux condenser and immersed in an oil bath held at 70° for 7 hr. An additional 2 ml $CH_2O$ and 2 ml $NHMe_2$ were added at 5 hr and again at 7 hr. After the reaction mixture had cooled to room temp., it was poured into a large beaker immersed in ice and made basic with conc. $NH_4OH$ while being stirred. After the mixture was cooled 1 hr on ice, the yellow ppt was collected by filtration and washed with $H_2O$. The solid was dissolved in hot aq. HCl, and the soln was filtered while hot. The filtrate was made basic by addition of conc. $NH_4OH$ with stirring and cooling on ice, affording a yellow ppt, which was collected by filtration after 1 hr cooling on ice. The ppt was washed with $H_2O$ and dried in air overnight to give 15.7 g 7-bromo-7-nitrogramine (**7**) as a yellow solid (91%): mp 181–182°. TLC, solvent B, $R_f = 0.12$. EIMS 10 eV *m/z* (rel. int.): 299, 297 $[M]^+$ (49, 51), 209, 207 $[M - NO_2 - NMe_2]^+$ (13, 12), 45 $[NHMe_2]^+$ (100). Found: C, 43.41; H, 4.31. $C_{11}H_{12}N_3O_2Br \cdot \frac{1}{2}H_2O$ requires: C, 43.02; H, 4.27. Recrystallization of 0.449 g **7** from aq. HCl gave yellow plates, which after filtration and drying in air overnight, yielded 0.362 g 7-bromo-5-nitrogramine hydrochloride (**7** · HCl) (72% reco-

very): mp 253°. TLC, solvent B, $R_f = 0.13$. $^1$H NMR (90 MHz, DMSO-$d_6$): $\delta$ 2.77 [6H, *s*, $\overset{+}{N}HMe_2$], 4.53 (2H, *s*, $CH_2$), 8.00 (1H, *d*, H-2, $J_{2,NH} = 2.8$ Hz), 8.21 (1H, *d*, H-6, $J_{6,4} = 2.2$ Hz), 8.96 (1H, *d*, H-4, $J_{4,6} = 2.2$ Hz), 11.00 and 12.50 [2H, 2 *br s*, $\overset{+}{N}HMe_2$ and NH], (DMSO-$d_6$–$D_2O$) 2.28 [6H, *s*, $NMe_2$], 3.69 (2H, *s*, $CH_2$), 7.60 (1H, *s*, H-2), 8.16 (1H, *d*, H-6, $J_{6,4} = 2.2$ Hz), 8.62 (1H, *d*, H-4, $J_{4,6} = 2.2$ Hz). EIMS 10 eV *m/z* (rel. int.) 299, 297 $[M - HCl]^+$ (52, 40), 255, 253 $[M - HCl - N(Me_2]^+$ (47, 46), 254, 252 $[M - HCl - HNMe_2]^+$ (16, 19), 45 $[NHMe_2]^+$ (65), 44 $[NMe_2]^+$ (100), 36 $[HCl]^+$ (30). Found: C, 39.43; H, 3.75; N, 12.43; Br, 23.84; Cl, 10.58. $C_{11}H_{12}N_3O_2Br \cdot HCl$ requires: C, 39.49; H, 3.92; N, 12.56; Br, 23.88; Cl, 10.60.

*7-Bromo-5-nitrogramine* (**8**), *3, 3'-bis(7-bromo-5-nitroindolymethyl)dimethylammonium iodide* (**9**) *and tetramethylammonium iodide* (**10**) *mixture.* To 250 ml MeI in a 500 ml round-bottomed flask was added in portions over 30 min with vigorous stirring 15.6 g 7-bromo-5-nitrogramine (**7**). The flask was stoppered and the yellow slurry was stirred at room temp. for 14 hr, after which the reaction mixture was evaporated to dryness at room temp. under red. pres. to give 23.0 g yellow powder (99% based on 7-bromo-5-nitrogramine methiodide, **8**): mp 228–229°. $^1$H NMR revealed the product to be a 4:1 mixture of 7-bromo-5-nitrogramine methiodide (**8**) and 3, 3'-bis(7-bromo-5-nitroindolylmethyl)dimethylammonium iodide (**9**) together with tetramethylammonium iodide (**10**): (90 MHz, DMSO-$d_6$) $\delta$2.97 (*s*, bis-Me), 3.10 (*s*, mono-Me), 3.14 (*s*, $\overset{+}{N}(Me)_4I^-$, increases on addition of **10**), 4.81 (*s*, mono-$CH_2$), 4.95 (*s*, bis-$CH_2$). 8.05, 8.28 and 9.00 (3 *m*, ArH), 12.69 (*br s*, NH).

*7-Bromo-5-nitro-3-indoleacetonitrile* (**11**). In a 1 l. three-necked Morton flask fitted with a thermometer, a stirrer and a reflux condenser was placed 22.0 g crude 7-bromo-5-nitrogramine methiodide (**8–10**), 11.0 g NaCN, 200 ml *n*-amyl alcohol, and 200 ml NaOAc buffer, which contained 6.0 g glacial HOAc and 13.6 g/l. NaOAc · $3H_2O$. After the reaction mixture was heated to 70° and stirred vigorously for 4 hr, it was allowed to cool to room temp. with continued stirring overnight. The resulting solid was separated from the two liquid phases by filtration, washed with $H_2O$ and dried in air overnight, giving 6.8 g 7-bromo-5-nitro-3-indoleacetonitrile (**11**), as a tan solid: mp 252.5–255°. TLC, solvent B, $R_f = 0.77$. IR $\nu_{max}^{Nujol\ mull}$ $cm^{-1}$ 2250 (C≡N str). $^1$H NMR (90 MHz, DMSO-$d_6$): $\delta$4.26 (2H, *s*, $CH_2$), 7.67 (1H, *d*, H-2, $J_{2,NH} = 2.7$ Hz, collapses to *s* with $D_2O$), 8.22 (1H, *d*, H-4, $J_{4,6} = 2.3$ Hz), 8.70 (1H, *d*, H-6, $J_{6,4} = 2.3$ Hz), 11.13 (1H, *br s*, NH, exchanges with $D_2O$). EIMS 10 eV *m/z* (rel. int.): 281, 279 $[M]^+$ (100, 98), 235, 233 $[M - NO_2]^+$ (25, 26), 154 $[M - NO_2 - Br]^+$ (24). Found: C, 42.71; H, 2.18; N, 14.79; Br, 28.59. $C_{10}H_6N_3OBr$ requires: C, 42.88; H, 2.16; N, 15.00; Br, 28.53. Additional product was obtained by separating the liquid phases and extracting the lower, aq. layer ×3 with 50 ml portions of *n*-amyl alcohol. The upper, organic layer and the *n*-amyl alcohol extracts were combined, washed successively (×3 each) with $H_2O$, 6 N HCl and $H_2O$ and then evaporated to drynesss under red. pres., yielding a yellow–orange residue, which was dissolved in hot EtOH, treated with activated charcoal, and filtered through Celite. Cooling and addition of 5 vols. $H_2O$ caused crystallization of an orange solid weighing 5.535 g. A portion of this (0.535 g) was purified by dissolving it in hot $Me_2CO$, filtering, cooling to room temp., adding $H_2O$, cooling on ice and collecting the resulting ppt by filtration. The solid was dried at 50° under vacuum for 6 hr, which gave 0.437 g (82% recovery) of a tan solid that was identical by mp, TLC, IR, $^1$H NMR and EIMS to 7-bromo-5-nitro-3-indoleacetonitrile (**11**) obtained by filtration of the rection mixture. The mp of a mixture of the two solids was not depressed. Combined yield of the two crude products was 74%.

*7-Bromo-5-nitro-3-indoleacetic acid* (**12**). In a 500 ml round-bottomed flask was placed 2.4 g 7-bromo-5-nitro-3-indoleacetonitrile (**11**) and 200 ml conc. HCl. The flask was fitted with a reflux condenser and the reaction mixture was heated at reflux with stirring for 6 hr, after which it was stirred at room temp. overnight. The resulting yellow ppt was collected by filtration, washed with $H_2O$ and dissolved in boiling $H_2O$ containing sufficient $Na_2CO_3$ to bring the pH of the soln to 9. The soln was treated with activated charcoal, filtered through Celite, cooled on ice, and acidified with conc. HCl while being stirred vigorously. After the yellow slurry was chilled on ice for 1 hr, the ppt was collected by filtration, washed with $H_2O$ and dried overnight at 60° under vacuum to give 2.35 g (92%) 7-Br-5-$NO_2$IAA (**12**) as a yellow solid: mp 243–246°. This was purified by recrystallization from MeOH–$H_2O$, which gave 1.90 g (81% recovery) 7-Br-5-$NO_2$IAA (**12**): mp 248.5° (blackens and foams). TLC, solvent B, $R_f = 0.74$. $^1$H NMR (90 MHz, DMSO-$d_6$): $\delta$ 3.81 (2H, *s*, $CH_2$), 7.56 (1H, *d*, H-2, $J_{2,NH} = 2.6$ Hz, collapses to *s* with $D_2O$), 8.18 (1H, *d*, H-4, $J_{4,6} = 2.4$ Hz), 8.57 (1H, *d*, H-6, $J_{6,4} = 2.4$ Hz), 11.93 and 12.27 (2H, 2 *br s*, NH and $CO_2H$, exchange with $D_2O$). EIMS 10 eV *m/z* (rel. int.): 300, 298 $[M]^+$ (54, 56), 255, 253 $[M - CO_2H]^+$ (85, 100), 209, 207 $[M - CO_2H - NO_2]^+$ (13, 17). Found: C, 40.40; H, 2.32; N, 9.25; Br, 26.58. $C_{10}H_7N_2O_4Br$ requires: C, 40.16; H, 2.36; N, 9.37; Br, 26.72.

*5-Amino-3-indoleacetic acid* (**13a**). *Method A*: In a 500 ml hydrogenation bottle, 0.350 g 7-Br-5$NO_2$IAA (**12**) was dissolved in 100 ml absolute EtOH. After the bottle was flushed with $N_2$, Raney nickel was added and the reaction mixture was shaken at room temp. under $H_2$ at 45 psi in a Parr apparatus for 3 hr. The catalyst was removed by filtration and washed ×3 with absolute EtOH. The combined filtrate and washings were evaporated to dryness at room temp. under red. pres., giving 5-$NH_2$IAA · HBr (**13a** · HBr) as a pale grey solid weighing 0.073 g (23%): mp 225° (blackens and foams, evacuated sealed tube). TLC, solvent B, $I_2$ visualization, $R_f = 0.34$. EIMS 10 eV *m/z* (rel. int.): 190 $[M - HBr]^+$ (84), 146 $[M - Br - CO_2H]^+$ (37), 145 $[M - HBr - CO_2H]^+$ (100), 80, 82 $[HBr]^+$ (33, 33). Found: C, 44.01; H, 4.20. $C_{10}H_{10}N_2O_2 \cdot HBr$ requires: C, 44.30; H, 4.09. Additional product was obtained as the free base by washing the catalyst with 2N $NH_4OH$ and evaporating the filtrate to dryness under red. pres. at 30° to give 5-$NH_2$IAA (**13a**) as a lavender solid weighing 0.116 g (52%): mp 242–244° (lit. [21, 33] 242–244°). TLC, solvent B, $I_2$ visualization, $R_f = 0.34$. $^1$H NMR (90 MHz, $D_2O$–NaOD): $\delta$ 3.60 (2H, *s*, $CH_2$), 4.73 (*s*, NH, $CO_2H$, $NH_2$, HOD), 6.76 (1H, *dd*, H-6, $J_{6,7} = 8.4$ Hz, $J_{6,4} = 2.4$ Hz), 6.99 (1H, *dd*, H-4, $J_{4,6} = 2.4$ Hz, $J_{4,7} = 0.8$ Hz), 7.16 (1H, *s*, H-2), 7.30 (1H, *dd*, H-7, $J_{7,6} = 8.4$ Hz, $J_{7,4} = 0.8$ Hz). EIMS 10 eV *m/z* (rel. int.): 190 $[M]^+$ (94), 146 $[M - CO_2]^+$ (22), 145 $[M - CO_2H]^+$ (100). EIMS and $^1$H NMR are identical to spectra for 5-$NH_2$IAA (**13a**) prepared previously from 5-$NO_2$IAA [21]. The free base was also obtained, along with 1 equivalent NaBr, in 83% yield when 1 equivalent NaOAc · $3H_2O$ was added to the reaction mixture. *Method B*: In a 500 ml hydrogenation bottle 0.150 g 7-Br-5-$NO_2$IAA (**12**) was dissolved in 1 ml 1 N KOH (2 equivalents) and 100 ml $H_2O$. The bottle was flushed with $N_2$ and 10% Pd on charcoal was added. The yellow reaction

mixture was shaken on a Parr apparatus under 45 psi $H_2$ at room temp. for 100 min and the catalyst was removed by filtration. The colorless filtrate was evaporated to dryness at 30° under red. pres., giving quantitatively, 5-$NH_2$IAA (**13a**) as a brown residue consisting of the K salt of **13a** and 1 equivalent KBr. This product was identical by TLC and $^1H$ NMR to the free base obtained in Method A. Similarly, a quantitative yield of the free base (identical by mp, TLC, EIMS, and $^1H$ NMR to the free base obtained from Method A) resulted when the reaction was run in absolute EtOH with 1 equivalent NaOAc · $3H_2O$, after filtration of the reaction mixture through Celite followed by evaporation of the filtrate to dryness under red. pres.

5-*Amino*-[7-$^2H$]*indole*-3-*acetic acid* (**13b**). In a 100 ml round-bottomed flask was placed 0.100 g 7-Br-5-$NO_2$IAA (**12**) dissolved in 10 ml $H_2O$ containing 0.032 g KOH, 0.12 g 10% Pd on charcoal and a magnetic follower. The flask was attached to a vacuum line, which was evacuated and filled with $^2H_2$ to atmospheric pres. The reaction mixture was stirred until it became colorless (2 hr), at which time no starting material could be detected by TLC (solvent B) and the $R_f$ of the product was 0.34. After the mixture was filtered through Celite, the solvent was removed under vacuum at room temp., producing a purple solid. The residue, crude 5-$NH_2$- [7-$^2H$]IAA (**13b**), was used in the synthesis of 5-$N_3$-[7-$^2H$]IAA (**14b**) without further purification. $^1H$ NMR (360 MHz, NaOD–$D_2O$): $\delta$3.56 (2H, *s*, $CH_2$), 6.76 (1H, *s*, H-6), 6.99 (1H, *s*, H-4), 7.15 (1H, *s*, H-2), 7.31 (0.29H, *d*, residual H-7, $J_{7,6}$ = 8.2 Hz).

5-*Amino*-[7-$^3H$]*indoleacetic acid* (**13c**). In a 25 ml round-bottomed flask was placed 0.100 g 7-Br-5-$NO_2$IAA (**12**) dissolved in 10 ml $H_2O$ containing 0.075 g KOH, a magnetic follower and 0.120 g 10% Pd on charcoal. 338 mCi $^3H_2$ was pumped into the vessel, followed by 24 ml $^1H_2$. The reaction was stirred until $H_2$ uptake ceased (3 hr), by which time the mixture was colorless. No starting material could be detected by TLC (solvent B). Unused $^3H_2$–$^1H_2$ was pumped into a vessel for disposal. The reaction mixture was filtered through Celite and the filtrate was evaporated to dryness under red. pres. at room temp. The resulting crude 5-$NH_2$-[7-$^3H$]IAA (**13c**) was used directly for synthesis of 5-$N_3$-[7-$^3H$]IAA (**14c**).

5-*Azidoindole*-3-*acetic acid* (**14a**). This compound was obtained from 5-$NH_2$IAA (13a) as previously described [21]. $^1H$ NMR (360 MHz DMSO-$d_6$): $\delta$3.63 (2H, *s*, $CH_2$), 6.82 (1H, *dd*, H-6, $J_{6,7}$ = 8.6 Hz, $J_{6,4}$ 2.2 Hz), 7.23 (1H, *d*, H-4, $J_{4,6}$ = 2.2 Hz), 7.29 (1H, *d*, H-2, $J_{2,NH}$ = 1.9 Hz), 7.38 (1H, *d*, H-7, $J_{7,6}$ = 8.6 Hz), irradiation at $\delta$7.38 caused *dd* at $\delta$6.82 to collapse to *d*.

5-*Azido*-[7-$^2H$]*indole*-3-*acetic acid* (**14b**). Crude 5-$NH_2$-[7-$^2H$]IAA (**13b**) was dissolved in 5 ml 80% aq. HOAc and cooled in an ice bath. To this was added 0.019 g $NaNO_2$ in 0.5 ml $H_2O$. To prevent photodecomposition, all manipulations after addition of $NaNO_2$ were carried out in the dark or under red light. After the soln was stirred for 15 min, 0.018 g $NaN_3$ in 0.5 ml $H_2O$ was added and stirring was continued for 5 hr at 0°. TLC of the reaction mixture showed that only one compound, $R_f$ = 0.91, was present. The solvent was removed under vacuum at room temp., leaving a residue which was suspended in 4 ml $H_2O$ and collected on a medium-frit sintered-glass funnel. This was dried *in vacuo* to yield 5-$N_3$-[7-$^2H$]IAA (**14b**) as tan crystals weighing 0.030 g (50%). $^1H$ NMR (360 MHz, DMSO-$d_6$): $\delta$ 3.63 (2H, *s*, $CH_2$), 6.82(1H, *br s*, H-6), 7.23 (1H, *s*, H-4), 7.29 (1H, *s*, H-2), 7.38 (0.32H, *d*, residual H-7, $J_{7,6}$ = 8.6 Hz), 11.05 (1H, *s*, $CO_2H$). FDMS isotope ratio analysis showed 17% undeuterated, 75% monodeuterated and 8% dideuterated product.

5-*Azido*-[7-$^3H$]*indole*-3-*acetic acid* (**14c**). To the crude 5-$NH_2$-[7-$^3H$]IAA (**13c**) prepared above was added 6 ml 80% HOAc. The reaction mixture was cooled in ice and 0.019 g $NaNO_2$ in 0.5 ml $H_2O$ was added. To prevent photodecomposition, all manipulations after addition of $NaNO_2$ were performed in the dark or under red light. After 15 min, 0.018 g $NaN_3$ in 0.5 ml $H_2O$ was added and the reaction mixture was stirred for 5 hr. The solvent was removed under vacuum at 30°. The residue was suspended in 4 ml $H_2O$ and collected on a medium-frit sintered-glass funnel, giving 5-$N_3$-[7-$^3H$]IAA (**14c**) weighing 0.012 g (17% from precursor **12**). Sp. act. of the product was 111 mCi/mmol, sp. act. of $^3H_2$ in the previous step being 314 mCi/mmol. After 1 month storage in 10 ml $C_6H_6$–EtOAc (2:1) at −20° in the dark, radiochromatography (solvent B) showed the product to be 98% radiochemically pure ($R_f$ = 0.89).

4-*Bromo*-1-*naphthaleneacetic acid* (**15**). Bromination of NAA [34] (**16a**) gave 4-BrNAA (**15**): mp 174–176° (lit. [34] 174.5–175.5°). TLC, solvent C, $R_f$ = 0.82. $^1H$ NMR (360 MHz, DMSO-$d_6$): $\delta$4.06 (2H, *s*, $CH_2$), 7.36 (1H, *d*, H-2, $J_{2,3}$ = 7.6 Hz), 7.68 (2H, *m*, H-6 and H-7), 7.83 (1H, *d*, H-3, $J_{3,2}$ = 7.6 Hz), 8.01 (1H, *d*, H-5 or H-8, $J$ = 8.0 Hz), 8.17 (1H, *d*, H-8 or H-5, $J$ = 7.3 Hz).

1-*Naphthaleneacetic acid* (**16a**). In a 500 ml hydrogenation bottle, 0.265 g 4-BrNAA (**15**) was dissolved in 100 ml absolute EtOH. To this was added, after flushing with $N_2$, 0.165 g NaOAc · $3H_2O$ (1 equivalent) and 0.100 g 10% Pd on charcoal, and the mixture was shaken at room temp., 45 psi $^1H_2$, for 90 min. The catalyst was removed by filtration through Celite and the filtrate was evaporated to dryness under red. pres. The residue was washed with $H_2O$ and recrystallized × 3 from $H_2O$, affording 0.045 g white needles (24%): mp 128–131° (lit. [35] 134.5–135.5°). TLC, solvent C, $R_f$ = 0.91. $^1H$ NMR (360 MHz, DMSO-$d_6$): $\delta$4.04 (2H, *s*, $CH_2$), 7.41–7.50 (2H, *m*, H-2 and H-3), 7.51–7.58 (2H, *m*, H-6 and H-7), 7.85 (1H, *dd*, H-4, $J_{4,3}$ = 7.5 Hz, $J_{4,2}$ = 1.7 Hz), 7.94 (1H, *dd*, H-5, $J_{5,6}$ = 7.1 Hz, $J_{5,7}$ = 1.9 Hz), 7.97 (1H, *dd*, H-8, $J_{8,7}$ = 8.3 Hz, $J_{8,6}$ = 1.4 Hz), broad irradiation of *m* at $\delta$7.41–7.50 caused collapse of *dd* at $\delta$7.85 to *d*. Broad irradiation of *m* at $\delta$7.51–7.58 caused collapse of *dd* at $\delta$7.97 to *d* and also caused collapse of *dd* at $\delta$7.94 to *d*. In samples of lower purity, the signals at $\delta$7.97 and $\delta$7.94 overlap.

[4-$^2H$]*Naphthalene*-1-*acetic acid* (**16b**). 4-BrNAA (0.134 g, **15**), 0.050 g 5% Pd on charcoal, 1 equivalent NaOAc · $3H_2O$, 50 ml absolute EtOH and a magnetic follower were placed in a 250 ml round-bottomed flask, which was then attached to a vacuum line. The mixture was frozen and the line was evacuated. $^2H_2$ was introduced at atmospheric pres. and the reaction was stirred for 3 hr. The catalyst was removed by filtration through a medium-frit sintered-glass funnel, 5 ml $H_2O$ was added to the filtrate, and the soln was evaporated under red. pres. at room temp. until crystallization occurred. The white needles were collected by filtration and air dried, yielding 0.050 g [4-$^2H$]NAA (53%). $^1H$ NMR (360 MHz, DMSO-$d_6$): $\delta$4.04 (2H, *s*, $CH_2$), 7.41–7.49 (2H, *m*, H-2 and H-3), 7.49–7.58 (2H, *m*, H-6 and H-7), 7.84 (*d*, 0.33 H, residual H-4), 7.95 [2H, pseudo *t* (2 *d* overlapping) H-5 and H-8]. EIMS isotope ratio analysis showed 27% undeuterated 70% monodeuterated, 3% dideuterated and 0.4% trideuterated product.

[4-$^3H$]*Naphthalene*-1-*acetic acid* (**16c**). To 0.029 g 4-BrNAA (**15**) dissolved in 5 ml absolute EtOH in a 10 ml round-bottomed flask was added 0.015 g 10% Pd on char-

coal, 0.013 g $NaOAc \cdot 3H_2O$ and a magnetic follower. The reaction vessel was attached to a vacuum line, the reaction mixture was frozen and the line was evacuated. 81.5 mCi $^3H_2$ (0.0318 ml) was pumped into the reaction vessel followed by 4.84 ml $^1H_2$. The reaction was warmed to room temp. and stirred for 3 hr. The $^3H_2$–$^1H_2$ mixture was pumped into another vessel for disposal and the reaction mixture was filtered through Celite, which was then washed with 10 ml absolute EtOH. To the combined filtrate and washings was added 5 ml $H_2O$. The resulting soln was concd under red. pres. until crystallization occurred. After collection by filtration and air drying, the colorless crystals of [4-$^3$H]NAA (**16c**) weighed 0.0075 g (39%). Radiochemical yield was 3.91 mCi and the sp. act. was 97.2 mCi/mmol. Radiochromatography (solvent C) showed the product to be >98% radiochemically pure, the radioactive spot having a $R_f$ = 0.87. After 1 month storage in absolute EtOH at −20° in the dark, no decomposition was detectable by radiochromatography.

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## NOTE ADDED IN PROOF

Shortly after this article was submitted an enzymic method for preparing small quantities of [5-$^3$H]IAA was published: Michalezuk, L. and Chisnell, J. R. (1982) *J. Labelled Cmpd. Radiopharmacol.* **19**, 21.