Tritiated Diimide: a Regio- and Stereo-selective Tritium-labelling Reagent

Manouchehr Saljoughian,*[a] Philip G. Williams,[a] Hiromi Morimoto,[a] David R. Goodlett[b] and Richard B. van Breemen[b]

[a] *National Tritium Labeling Facility and Structural Biology Division, Lawrence Berkeley Laboratory 75-123, University of California, Berkeley, CA 94720, USA*
[b] *Department of Chemistry, North Carolina State University, Raleigh, NC 27695-8204, USA*

We report the synthesis of high specific activity tritiated diimide as a valuable reagent for the addition of tritium across symmetrical multiple bonds, and illustrate its application by regiospecific tritiation of a series of substituted cinnamates and an alkenyl organophosphonate.

We have synthesized and used tritiated diimide (HN=NT) at high specific radioactivity (A_s). The reagent effected double-bond reductions in the presence of other functionalities, including nitro, bromo, azido, methoxy, ester and phosphonate groups. The ability to hydrogenate multiple bonds in the presence of these functional groups will permit much simpler chemistry in many tritium-labelling reactions, and will often allow the tritiation procedure to be the final synthetic step in the production of a labelled compound.†

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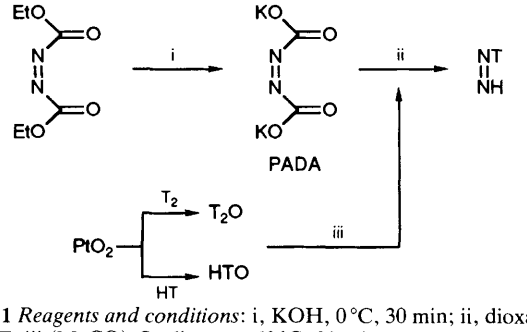
Diimide is a facile, selective and stereospecific hydrogenation reagent,[1] and its action is unaffected by sulfur-containing molecules,[2a] in contrast to metal-catalysed hydrogenation systems. It reduces symmetrical double bonds, but leaves unsymmetrical or more polar double bonds and carbon–halogen bonds intact, and this is the source of its selectivity.[2a] Reduction results in stereospecific *cis* addition,[1c] and product studies show that hydrogen insertion occurs from the less hindered side of the molecule.[2b] These observations have been used to propose a concerted or synchronous dihydrogen-transfer mechanism, involving a cyclic transition state.[1c,3] A convenient source of diimide is from the acid-catalysed decomposition[4] of potassium azodicarboxylic acid[5] (PADA) in the presence of the substrate.

Dideuteriodiimide[1c] has been used recently for the stereospecific deuterium labelling of substituted cyclopropane compounds.[6] The authors went to great lengths to exclude proton sources from all the reactants, and they achieved 96% incorporation of deuterium across the double bond. Tritiated diimide has previously been reported for labelling steroids,[7a] fatty acids,[7b] and amino acids[7c] at specific radioactivities up to 0.037 GBq mmol^{-1} (*cf.* theoretical maximum A_s is 2128 GBq mmol^{-1}). While these previous studies[7] reported at most one tritiated molecule in every 60000, the current work leads to an average of one tritiated molecule in every six. In most deuterium and tritium studies, a primary kinetic isotope effect on the rate of the diimide reduction has been suspected, but could not be quantitated.[6,7]

We illustrate our simple tritiation technique with the following general procedure (Scheme 1), adapted from an empirical study of reaction conditions.[8] Precursor (10–20 mg, *ca.* 50 μmol) and PADA (50 mg, *ca.* 250 μmol) were added to a 5 ml, flame-dried round-bottomed flask equipped with a side-arm and a small stirring bar. The materials were thoroughly stirred, and the flask was rigorously evacuated. In a separate flask, tritiated acetic acid (22 μl, 380 μmol) was prepared by treating acetic anhydride with tritiated water for 30 min at 60 °C, and the resulting acid was dissolved in dioxane (sodium dried, 0.5 ml). High-level tritiated water was prepared from either carrier-free tritium gas or a 10% mixture of ^{3}H in ^{1}H (Scheme 1).[9] The acetic acid–dioxane solution was transferred through a long needle to the reaction vessel. The reaction vessel was then isolated and the pressure maintained at *ca.* 60 kPa with nitrogen gas, while the mixture was stirred at ambient temperature for 18 h. The solvent and the excess of acetic acid were then removed under vacuum, and methanol (1 ml) was added and evaporated. The reaction products were then dissolved in [$^2H_6$]benzene (1 ml), and filtered through glass wool to remove potassium acetate. Aliquots of the filtrate were used for liquid scintillation counting, radio-HPLC, and ^{3}H and ^{1}H NMR analyses.



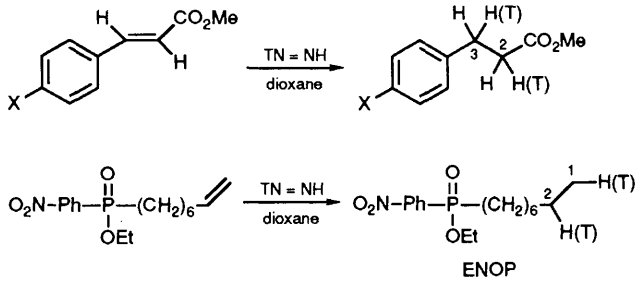
Scheme 1 *Reagents and conditions*: i, KOH, 0 °C, 30 min; ii, dioxane, $MeCO_2T$; iii $(MeCO)_2O$, dioxane, 60 °C, 30 min

Optimum reaction conditions and parameters for isolation of products were established using D_2O as the isotope source in hydrolysis of acetic anhydride. ^{1}H, ^{2}H and ^{3}H NMR spectroscopic techniques[10] were used to confirm the specificity and selectivity of the addition of tritium or deuterium across the double bond of a range of substituted methyl cinnamates, without reduction of nitro, bromo, azido or ester functional groups. Radio-HPLC analyses confirmed the specific activity and radiochemical purity of tritiated products, and results derived from the NMR and HPLC analyses of the reaction products are given in Table 1. Careful ^{1}H and ^{2}H NMR analyses of deuteriated products indicated that only 50–55% of the theoretical deuterium was incorporated in the desired positions, with none added elsewhere, and the deuterium content of these samples was corroborated by mass spectrometry. A large number of reaction conditions were tested in an attempt to eliminate the protium incorporation during deuterium addition, without success. Previous dideuteriodiimide reactions[6] have achieved 96% deuterium incorporation, but we note that the chemical scale was *ca.* 2000 times larger.

A similar, but larger, effect was observed in tritiation reactions (Table 1), with only 17–25% of the expected tritium incorporated into the product. The consistency of our results for many deuterium reactions, and a number of tritium reactions (with different tritium : hydrogen ratios) suggests to us that a significant primary kinetic isotope effect influences the formation of diimide from PADA.

The alkyl region of the NMR spectra of crude products from three different diimide reductions of 4-nitro methyl cinnamate are shown in Fig. 1(*a*)–(*e*). The ^{2}H and ^{3}H NMR spectra of all the products in Table 1 were similar to those shown here, and revealed uneven addition of deuterium or tritium across the double bond [Fig. 1(*b*)–(*d*)]. These differences are quantitated in Table 1, with 6–12% more deuterium at the 3- *vs.* the 2-position, and 17–21% more tritium at the 3- *vs.* the 2-position. These differences reflect slightly different reactivity at each end of the double bond, presumably as a result of the functional group substitution on these carbon atoms (*i.e.*

Table 1 Tritiated diimide reduction of a series of substituted methyl cinnamates[a]



ENOP

Compound R-X	^{3}H (%)	Yield (%)	A_s/GBq mmol^{-1}	(%) of Theory	δ 2-^{3}H	δ 3-^{3}H	3-^{3}H *vs.* 2-^{3}H	3-^{2}H *vs.* 2-^{2}H
R-NO_2[b]	100	59	363	17	2.11	2.48	1.18	1.06
R-NO_2[c]	10	62	52	24	2.11	2.48	1.18	1.06
R-Br	10	66	48	23	2.17	2.52	1.17	1.06
R-N_3	10	63	37	17	2.23	2.62	1.20	1.08
R-OMe	—	45	—	—	—	—	—	1.12
ENOP[d]	10	—	—	—	0.88	1.23	1.21	—

[a] All deuteriated products had 50–55% of the theoretical ^{2}H incorporation. [b] For use of 100% ^{3}H the maximum theoretical A_s = 2128 GBq mmol^{-1}. [c] For use of 10% ^{3}H in ^{1}H the maximum theoretical A_s = 212.8 GBq mmol^{-1}. [d] The NMR data give the ratio of 2-^{3}H *vs.* 1-^{3}H for this substrate.

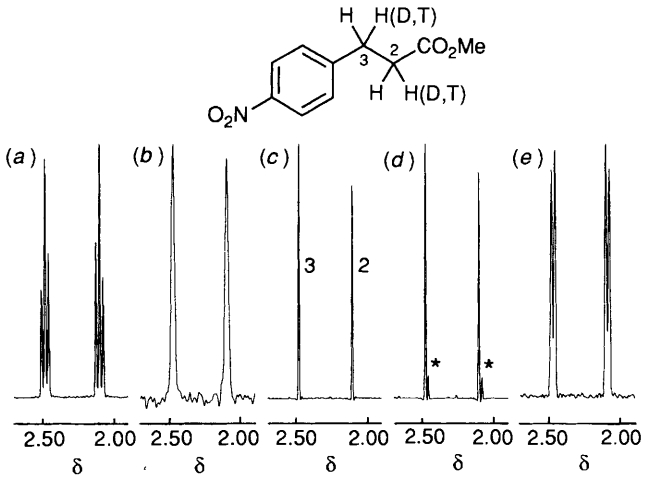


Fig. 1 NMR spectra of the alkyl region (δ 2.70–1.90) of the products from diimide reductions of methyl 4-nitrocinnamate. (*a*) 300 MHz ^{1}H NMR spectrum of the tritiated product in (*c*). (*b*) Proton-decoupled 46 MHz ^{2}H NMR spectrum of the deuteriated product. (*c*) Proton-decoupled 320 MHz ^{3}H NMR spectrum of the product of the 10% ^{3}H/^{1}H reaction. (*d*) Proton-decoupled 320 MHz ^{3}H spectrum of the product of the 100% ^{3}H reaction. (*e*) Continuously proton-decoupled 320 MHz ^{3}H double quantum filtered (DQF) spectrum of the product in (*d*). All spectra were acquired on an IBM Instruments AF-300 NMR spectrometer, with 8 K data points, and the FID was Gaussian multiplied (LB = −1, GB = 0.10) and zero-filled to 16 K points before Fourier transformation. Samples for proton and tritium NMR analyses were dissolved in C_6D_6, and deuterium samples were in C_6H_6. The DQF spectrum shows a magnitude calculation display of signal intensities.

ester function *vs.* phenyl ring). We believe this is the first observation of such a phenomenon for a diimide reaction, and we note that the effect is greater for tritium than deuterium addition. The classical concerted mechanisms for diimide reductions give no indication of such effects, although the formation of an unsymmetrical transition state is not excluded. A notable feature of Fig. 1(*d*) is the appearance of small peaks (*) upfield of each large tritium signal, and these arise from molecules which contain two tritium atoms. The true signals are doublets at each chemical shift, with the downfield line of the doublet obscured in each case by the singlet of the more abundant mono-tritiated species. An estimation of the ratio of $^3H_1$ *vs.* $^3H_2$ molecules may be made from the integrals of these signals, and this indicates that *ca.* 8% of the tritiated molecules have two tritium atoms (*i.e.* 15% of the radioactivity is in these molecules). The large singlet signals may be suppressed and the doublets (J_{TT} 7.81 Hz) revealed by application of a double quantum coherence NMR sequence,[11] and the resultant spectrum is shown in Fig. 1(*e*).

Labelling of ethyl *O*-*p*-nitrophenyl oct-7-enylphosphonate[12a] quantitatively yielded the pure, specifically tritiated product (ENOP, see Table 1) with the tritium in a biologically stable position. The specific activity of this product was not determined so the incorporation percentage is unknown, but the substituted end of the double bond was more highly tritiated. Alkyl phosphonates, such as ENOP, are good inhibitors of juvenile hormone (JH) esterase,[12b] and the tritium-labelled ENOP is currently being used in the labelling of active-site residues in JH esterase.

Some interesting points arise from our evolution of a facile tritium-labelling process. Despite our best efforts, at common tritiation scales (0.05–0.5 mmol substrate) tritium incorporation is substantially below theoretical levels, and we note that the unwanted incorporation of hydrogen is greater for tritiation reactions than in deuteriations. Neither tritium nor deuterium are incorporated equally across double bonds in the reduction of substituted methyl cinnamates, or the terminal double bond of an alkenyl phosphonate. These observations suggest the possibility of isotope effects on both the formation of the diimide reagent, and in the transfer of hydrogen to the target molecule. It is interesting to note that deuterium and tritium labelling allow observation of slight differences in bond polarization: *i.e.* very polar (unsymmetrical) multiple bonds are not reduced by diimide, but we have shown that the two ends of 'symmetrical' double bonds may be differentially labelled.

We have demonstrated the use of tritiated diimide at high specific activity as a reagent for facile stereospecific and selective labelling of organic compounds containing both symmetrical carbon–carbon double bonds *and* potentially reducible functionalities such as aryl nitro, bromo, azido, alkyl ester and phosphonate groups. We note especially that the azido group was not reduced under the diimide hydrogenation conditions, and therefore, our procedure provides a simple synthesis of tritium-labelled photoaffinity probes bearing an azido group as a nitrene precursor.[13] The experimental manipulations were simple and gave tritiated products at 37–370 GBq mmol^{-1} specific activity in one-pot syntheses.

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