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Research Article

Development of a photoaffinity label for respiratory syncytial virus inhibitors

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Summary

The syntheses of tritiated and radio-iodinated respiratory syncytial virus inhibitor photoaffinity labels are reported. ^{3}H-1-Isopropyl-3-(1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-1H-benzoimidazol-2-yl)methyl)-1,3-dihydrobenzoimidazol-2-one (**1**), was prepared in two steps by the initial tritiation of *N*-isopropenyl-2-benzimidazolone followed by coupling to 2-chloromethyl-1-[2-(3-methyl-3H-diazirin-3-yl)ethyl]-1H-benzoimidazole. The mono and diiodo-radio-iodinated derivatives of 1-(4-hydroxybenzyl)-3-(1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-2,3-dihydro-1H-benzo-imidazol-2-ylmethyl)-1,3-dihydrobenzoimidazol-2-one (**2, 3**), were prepared by the radio-iodination of the corresponding phenol with Na^{125}I and iodogen. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: photoaffinity label; radioiodination; RSV inhibitor

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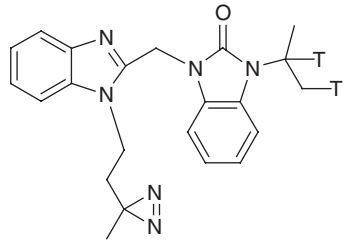
Introduction

Respiratory syncytial virus (RSV) has been recognized as a leading cause of virus-induced lower respiratory tract disease in infants, and children.[1–3] Continuing studies have also shown that RSV is a significant etiology agent in other populations especially the elderly and the immunocompromised.[4–8]

This manuscript describes the design and synthesis of a photoaffinity label that was utilized to confirm the identity of the virus protein targeted by this class of RSV inhibitors and to label the specific amino acid residues intimately involved in the binding interaction. The results have delineated a binding site for this class of compounds and provided insight into the mode of action at a molecular level.[9]

The design of the photoaffinity probe was predicated on the convergence of structure-activity relationships (SAR) developed throughout the program with the physicochemical properties of a suitable labeling element. The diazirine moiety was selected as the preferred photo-activated element because of the powerful labeling associated with carbene derivatives and that the SAR developed indicated tolerance for small heterocycles at the terminus of the alkyl side chain.[10] As a consequence, the diazirine derivative **8** was identified as a structural element that met these specifications and was readily



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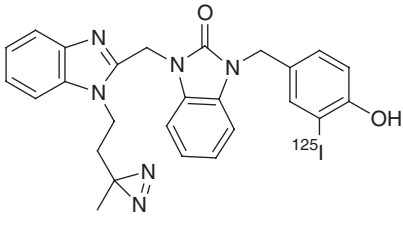

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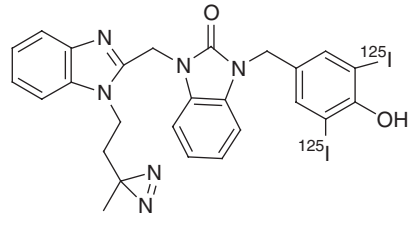

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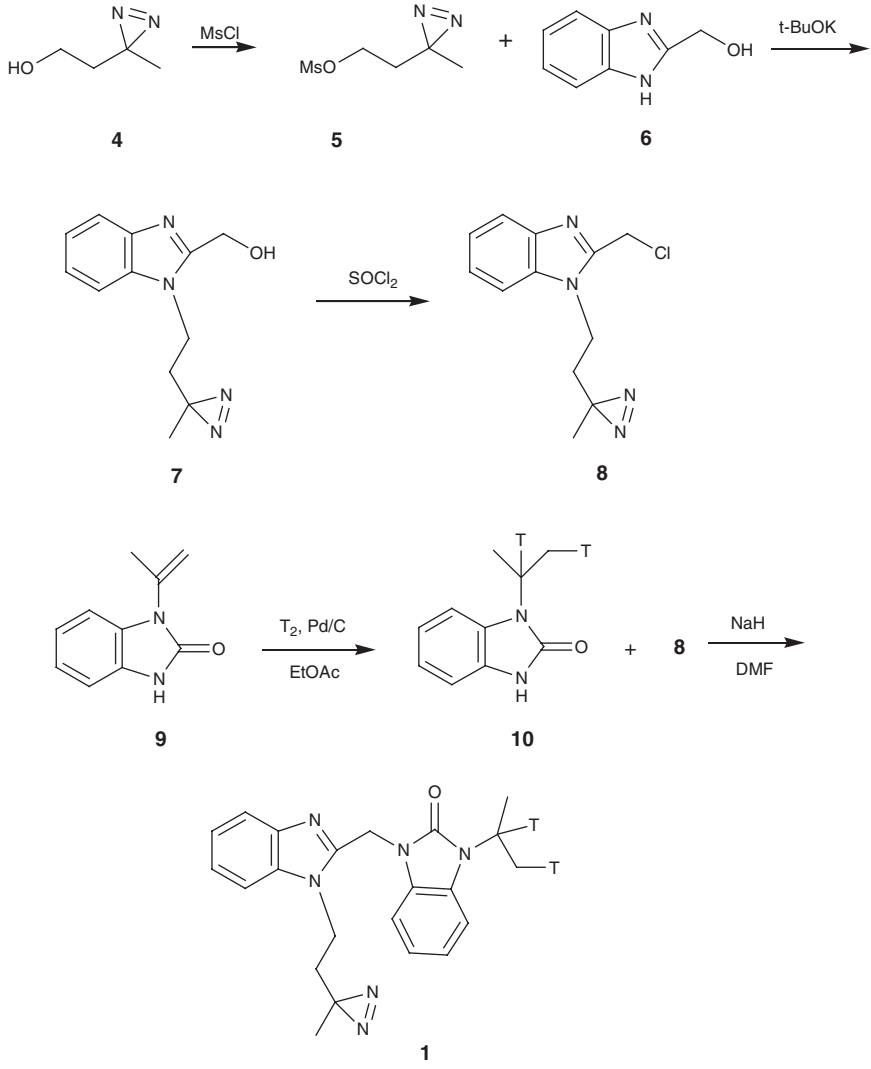
Figure 1. Structures of photoaffinity probes 1, 2 and 3

derivatized at a late stage to afford either the tritiated derivative **1** or the radio-iodinated derivatives **2** and **3** (Figure 1).

Results and discussion

^{3}H-labeled-1-Isopropyl-3-(1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-1H-benzo-imidazol-2-yl)methyl)-1,3-dihydrobenzoimidazol-2-one **(1)** was prepared in two steps as shown in Scheme 1. Mesylation of



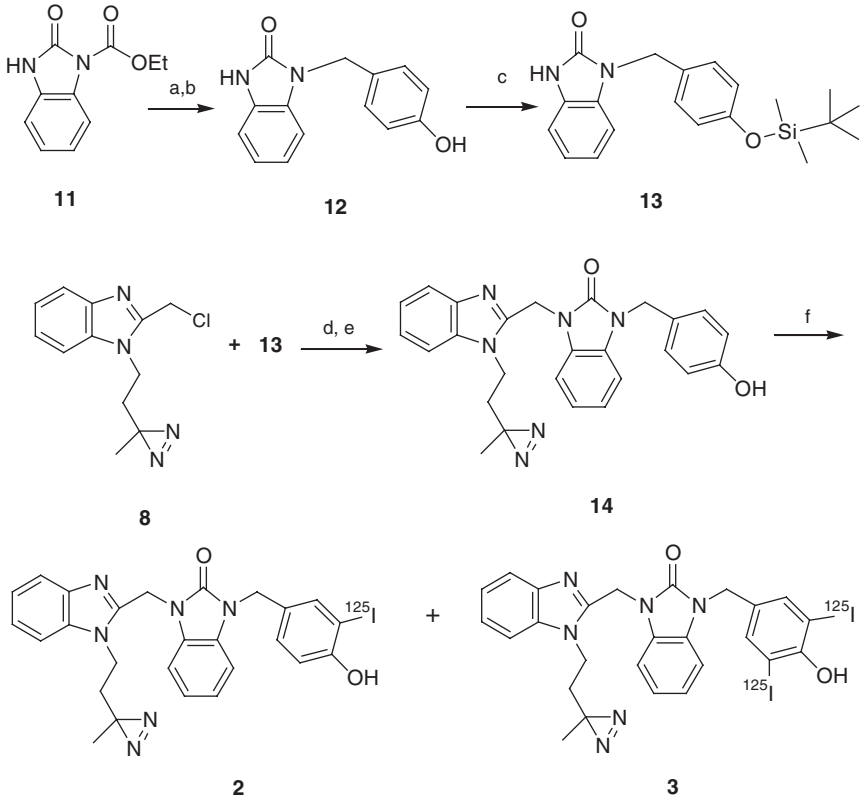
Scheme 1. Synthesis of ^{3}H-labeled 1-Isopropyl-3-(1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-1H-benzoimidazol-2-yl)methyl)-1,3-dihydrobenzoimidazol-2-one, (1)

3,3-azobutanol[11] (**4**) provided **5** which was exposed to 2-benzimidazolemethanol (**6**) in the presence of potassium tert-butoxide to afford the *N*-alkylated derivative **7**. Chlorination of the alcohol moiety of **7** using thionyl chloride provided the benzimidazole derivative **8**. *N*-isopropenyl-*N*-benzimidazolone (**9**) was tritiated under standard conditions to yield approximately 7 Ci of ^{3}H-labeled *N*-isopropyl-*N*-benzimidazolone (**10**). Alkylation of the benzimidazolone **10** with the diazirine **8** was accomplished by using sodium hydride as the base in DMF as solvent to afford the ^{3}H-labeled photoaffinity probe **1**.

The specific activity of **1** was found to be 150 Ci/mmol. This value implies 5.2 tritium atoms per molecule. ^{3}H NMR analysis of this material clearly indicated extensive labeling in the methyl position of the isopropyl group. The ratio of the methyl tritium to the methine tritium was 11.9:1 in the proton-decoupled spectrum, and 12.0:1 in the proton-coupled spectrum. (Proton decoupling can differentially change the tritium signal intensity through NOE if not carefully done). Theoretically, these values should be 1:1, however unequal addition of tritium across double bonds is a well known phenomenon. Williams and coworkers have published a detailed account of the use of modern ^{3}H NMR techniques to analyze these complex mixtures.[12]

Binding studies with **1** demonstrated that the label was associated with the RSV F protein, but the specific activity of the ^{3}H material was insufficient to conduct subsequent mapping studies. These results prompted us to prepare a radio-iodinated photoaffinity label and thereby take advantage of the higher specific activity available with mono or di-radio-iodinated probes.

The ^{125}I labeled radio-iodinated phenols were synthesized as depicted in Scheme 2. Ethyl 2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxylate (**11**) was alkylated with 4-(chloromethyl)phenyl acetate using *t*-butylimino-tri(pyrrolidino)phosphorane (BTPP) as the base in the presence of tetrabutylammonium bromide. The crude product was doubly deprotected by exposure to 4 N sodium hydroxide in methanol and the liberated phenol **12** protected as the *tert*-butyldimethylsilyl ether **13**. Alkylation of **13** with the diazirine derivative **8** using BTPP as the base followed by removal of the silyl protecting group with TBAF in THF provided phenol **14**. Radio-iodination of the **14** was accomplished by treatment with Na^{125}I and iodogen. Binding studies were conducted with both **2** and **3** and demonstrated that both labels behaved similarly and were associated with the RSV F protein. Subsequent mapping studies were then conducted with **3** to take advantage of the higher



Reagents: a, 4-(chloromethyl)phenyl acetate, BTPP, $(Bu)_4N^+Br^-$, CH_2Cl_2; b, NaOH, CH_3OH; c, *t*-butyldimethylsilyl chloride, imidazole, DMF; d, BTPP, CH_2Cl_2; e, TBAF; f, $Na^{125}I$, iodogen.

Scheme 2. Synthesis of ^{125}I labelled 2 and 3

specific activity of **3**. The results of these studies have recently been reported in part and a complete manuscript will be published elsewhere.[9]

Materials and methods

$Na^{125}I$ was obtained from both Nordion International Inc and DuPont/NEN. All other reagents were obtained from Aldrich unless otherwise noted and were either ACS grade or the highest quality material commercially available. HPLC purification and analysis was performed on a Rainin Dynamax HPLC system consisting of two SD-200 pumps, a Rainin UV-I detector and an *INUS* γ-RAM radioactive flow-through

detector. A Biodex Medical Systems Atomlab™ 100 Dose Calibrator and a Wallac Model 1409 liquid scintillation counter were used for radioactivity measurements.

High performance liquid chromatography

Method A. In this method samples are loaded on to an R_xCN column (4.6 × 250 mm) with a mobile phase of 10% CH_3CN and 90% 0.1% TFA at a flow rate of 1.0 ml/min. The UV-1 detector was set at 265 nm. In this system, *N*-isopropenyl-2-benzimidazolone (**9**) has an R_t of approximately 28.7 min and *N*-isopropyl-2-benzimidazolone (**10**) has an R_t of approximately 33.5 min.

Method B. In this method samples are loaded on to an R_xCN column (4.6 × 250 mm) with a mobile phase of 45% CH_3CN and 55% 0.1% TFA at a flow rate of 1.0 ml/min. The UV-1 detector was set at 265 nm. In this system, *N*-isopropyl-2-benzimidazolone (**10**) has an R_t of approximately 4.7 min, *2*-chloromethyl-1-[2-(3-methyl-3H-diazirin-3-yl)ethyl]-1H-benzoimidazole (**8**) has an R_t of approximately 7.1 min and the desired product **1** has an R_t of approximately 12.6 min.

Method C. In this method samples are loaded onto a Jupiter C18 column (4.6 × 250 mm, 5 μ 300 Å) with a mobile phase of 35% CH_3CN and 65% 0.1% TFA at a flow rate of 1.0 ml/min. The UV-1 detector was set at 271 nm. In this system, **2** has an R_t of approximately 24 min while **3** has an R_t of approximately 37 min.

Experimental

Methanesulfonic acid 2-(3-methyl-3H-diazirin-3-yl)ethyl ester, **5**: To a stirred solution of alcohol **4**[11] (1.01 g, 10 mmol) in dichloromethane (15 ml) at 0°C was added methanesulfonyl chloride (1.2 g, 10.5 mmol) followed by triethylamine (1.52 g, 15 mmol). After stirring for 2 h at 0°C the reaction was diluted with water and the product extracted with diethyl ether. Purification by flash column chromatography gave 0.95 g (49% yield) of **5** as an oil which was used in the next step.

{1-[2-(3-Methyl-3H-diazirin-3-yl)-ethyl-1H-benzoimidazol-2-yl}-methanol, **7**: To a solution of 2-benzimidazolemethanol (**6**) (Aldrich, 1.0 g, 6.8 mmol) in anhydrous THF (20 ml) was added potassium *t*-butoxide (1 M in THF, 6.8 ml, 6.8 mmol) at room temperature. After stirring for 10 min, mesylate **5** (1 g, 5.2 mmol) was added and the

suspension was stirred at room temperature for 18 h. As the reaction was incomplete, the mixture was heated to 70°C for 5 h. The solvent was evaporated. The residue was diluted with aqueous saturated sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$), filtered, and evaporated. Flash column chromatography (gradient, 1:1 EtOAc/hexane to 2:1 EtOAc/hexane to 10:1 EtOAc/methanol) gave 642 mg (41% yield) of **7** as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 3H), 1.86–1.91 (m, 2H), 3.97 (br s, 1H), 4.10–4.22 (m, 2H), 4.95 (s, 2H), 7.23–7.31 (m, 3H), 7.68–7.71 (m, 1H). IR (KBr, cm^{-1}) 3153, 1469, 1038, 748. MS m/e 231 $[M+H]^+$. Anal. Calcd. for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33; Found C, 62.43; H, 6.09; N, 24.50.

2-Chloromethyl-1-[2-(3-methyl-3H-diazirin-3-yl)ethyl]-1H-benzoimidazole, **8**: To a solution of alcohol **7** (230 mg, 1 mmol) in dichloromethane was added thionyl chloride (238 mg, 2 mmol). The reaction mixture was stirred at room temperature for 30 min before evaporating the solvent. The residue was diluted with ethyl acetate, washed with aqueous saturated sodium bicarbonate, dried ($MgSO_4$), filtered and the solvent removed by evaporation. The crude product was purified by flash column chromatography to give 210 mg (85% yield) of **8** as an oil which solidified upon standing. ^{1}H NMR ($CDCl_3$) δ 1.02 (s, 3H), 1.92–1.97 (m, 2H), 4.18–4.23 (m, 2H), 4.94 (s, 2H), 7.28–7.36 (m, 3H), 7.75–7.79 (m, 1H). IR (KBr, cm^{-1}) 1467, 753. MS m/e 249 $[M+H]^+$. Anal. Calcd. for $C_{12}H_{13}ClN_4$: C, 57.95; H, 5.27; N, 22.53; Found C, 57.68; H, 5.24; N, 22.60.

1-Isopropenyl-2-benzimidazolone, **9**: Compound **9** was prepared as described in the literature.[13] ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3 H), 5.23 (s, 1 H), 5.39 (d, $J = 1.0$ Hz, 1 H), 7.05–7.11 (m, 4 H), 9.69 (s, 1 H). IR (KBr, cm^{-1}) 1694, 1479, 1386, 740. MS m/e 175 $[M+H]^+$.

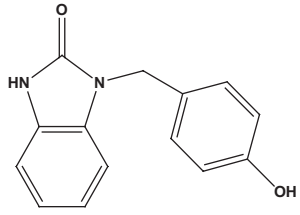
^{3}H-N-isopropyl-2-benzimidazolone, **10**: A sample of *N*-isopropenyl-2-benzimidazolone (**9**), (10.2 mg, 58.5 μm) was dissolved in EtOAc (1.5 ml) and the solution frozen in liquid N_2, and then thawed under vacuum to degas the solution prior to tritiation. The process was repeated twice prior to the addition of the 10% Pd/C catalyst (15.9 mg). The tritiation was allowed to proceed under 740 mm of T_2 gas for 6 h. After 6 h, the excess tritium was returned to the uranium bed and the solvent removed under vacuum. Labile tritium was removed by dissolving the residue in EtOAc (1 ml) and evaporating under vacuum (2x). ^{3}H-*N*-isopropyl-2-benzimidazolone, (**10**), (7.5 Ci, 150 Ci/mmol) was then dissolved in EtOAc (7 ml) for use in the next reaction. The radiochemical purity of **10**, was >99% (HPLC Method A).

^{3}H-1-Isopropyl-3-(1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-1H-benzoimidazol-2-ylmethyl)-1,3-dihydrobenzoimidazol-2-one, **1**: *The following procedure was conducted under red light*. A solution of **10** in EtOAc, (2 Ci, 16.7 μm, 1.85 ml) was evaporated to near dryness under a stream of N_2 and then placed under vacuum for 10 min. To this was then added DMF (0.5 ml) and the solution transferred to an amber colored 5 ml reaction vial and to this was added NaH (60%, 6.0 mg, 14.2 μm). The mixture was stirred for 0.5 h, after which time **8**, (4 mg, 16 μm) was added and the reaction allowed to stir at room temperature for 16 h. The reaction was quenched with saturated $NaHCO_3$, rinsed with H_2O and extracted with Et_2O. The Et_2O layer was dried by passage through a short column of $MgSO_4$. The solution was concentrated to dryness under a stream of N_2 and immediately dissolved in 5 ml of HPLC mobile phase (45% CH_3CN and 55% 0.1% TFA). A 100 mCi sample of crude **1** was purified via HPLC (Method B) to yield **1** (70 mCi). No radioactive impurities were found in the final purified product. The specific activity was determined via HPLC. In this procedure, a radioactive sample was applied to the column, and the mass was determined by comparison of the UV absorbance to a standard curve, and the total radioactivity was measured via liquid scintillation counting. The specific activity of **1** was found to be 150 Ci/mmol.

Stability studies of the ^{3}H-photoaffinity label **1** were conducted in EtOH and HPLC mobile phase at 0°C for 2 weeks in amber colored screw top reaction vials. At the end of 2 weeks, it was found that 4 radiolabeled impurities representing 9% of the total radioactivity were found in the sample stored in EtOH, whereas no radiolabeled impurities were found in the sample stored in the HPLC mobile phase. Samples of **1** for subsequent biological studies were thus kept in the HPLC mobile phase until immediately prior to the intended use when the mobile phase was removed by lyophilization and then immediately redissolved in EtOH.

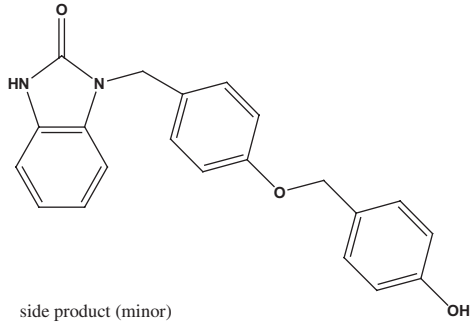
1-[4-(tert-Butyl-dimethylsilanyloxy)-benzyl]-1,3-dihydro-benzoimidazol-2-one, **13**: A mixture of **11**[14] (1.55 g, 7.5 mmol), 4-(chloromethyl)phenyl acetate (1.52 g, 8.25 mmol), BTPP (Fluka, *t*-butyliminotri(pyrrolidino)phosphorane, 2.81 g, 9.0 mmol), and tetrabutylammonium bromide (2.9 g, 9.0 mmol) in dichloromethane (20 ml) was stirred at room temperature for 2 h. The solvent was evaporated off and the residue was dissolved in ethyl acetate, washed with water, dried ($MgSO_4$), filtered and evaporated. This residue was dissolved in methanol (30 ml) and to this solution was added 4 N NaOH (5 ml).

The mixture was stirred at room temperature for 30 min. The methanol was evaporated off and the aqueous layer acidified with concentrated HCl and extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$), filtered and evaporated. Purification of **12** was attempted using flash column chromatography (gradient 1:1 EtOAc/hexanes to straight EtOAc to 10:1 EtOAc/MeOH) and gave 680 mg of the **12** containing 30% of an impurity (structure below). The product was used in the next step without further purification.



12

desired product (major)



side product (minor)

The crude phenol **12** (680 mg), *tert*-butyldimethylsilyl chloride (467 mg, 3.10 mmol, 1.1 eq), and imidazole (561 mg, 8.49 mmol, 3 eq) were stirred together in DMF (10 ml) at 75°C for 18 h. The reaction was incomplete. After cooling to room temperature, additional *tert*-butyldimethylsilyl chloride (467 mg, 3.10 mmol) and imidazole (561 mg, 8.49 mmol) were added and the reaction mixture was stirred at room temperature for 1 h. The mixture was diluted with ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash column chromatography (gradient, 1:4 EtOAc/hexanes to 1:1 EtOAc/hexanes) to give 335 mg of **13** as a white solid. MS m/e 355 $[M+H]^+$. ^1H NMR ($CDCl_3$) δ 0.17 (s, 6H), 0.96 (s, 9H), 5.01 (s, 2H), 6.77–6.80 (m, 2H), 6.87–6.90 (m, 1H), 6.98–7.11 (m, 3H), 7.21 (d, $J=8.7$ Hz, 2H), 9.34 (s, 1H). Anal. Calcd. for $C_{20}H_{26}N_2O_2Si$: C, 67.76; H, 7.39; N, 7.90, Found: C, 67.39; H, 7.28; N, 7.63.

1-(4-Hydroxybenzyl)-3-{1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-2,3-dihydro-1H-benzoimidazol-2-ylmethyl}-1,3-dihydro-benzoimidazol-2-one, **14**: A mixture of **8** (216 mg, 0.87 mmol), **13** (308 mg, 0.87 mmol), and BTPP (Fluka, *t*-butylimino-tri(pyrrolidino)phosphorane, 352 mg, 1.13 mmol) was stirred in dichloromethane (5 ml) for 1 h at room

temperature. The solvent was evaporated off and the residue diluted with ethyl acetate, washed with water, brine, dried ($MgSO_4$), filtered and evaporated. Flash column chromatography (gradient, 1:4 EtOAc/hexane to 1:1 EtOAc/hexane) gave the coupling product, as the silyl ether which was immediately used in the next step. MS m/e 567 $[M+H]^+$.

To the silyl ether was added 1.3 ml of 1 M TBAF (tetrabutylammonium fluoride) solution in tetrahydrofuran. The mixture was stirred at room temperature for 1 h, diluted with ethyl acetate and diethyl ether, washed with water, dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (gradient, 1:1 EtOAc/hexanes to straight EtOAc) to give 232 mg (59%) of **14** as a white solid. ^1H NMR ($CDCl_3$) δ 1.00 (s, 3H), 1.55 (t, $J=7.8$ Hz, 2H), 4.43 (t, $J=7.8$ Hz, 2H), 5.00 (s, 2H), 5.60 (s, 2H), 6.81 (d, $J=8.1$ Hz, 2H), 6.89–6.92 (m, 1H), 6.99–7.02 (m, 2H), 7.21 (d, $J=8.1$ Hz, 2H), 7.31–7.55 (m, 3 H), 7.50–7.60 (m, 1H), 7.84–7.89 (m, 1H). IR (KBr, cm^{-1}) 2925, 1708, 749.

1-(4-Hydroxy-3,5-diiodo-benzyl)-3-{1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-2,3-dihydro-1H-benzoimidazol-2-ylmethyl}-1,3-dihydrobenzoimidazol-2-one, **3a**: To a solution of iodogen (19.2 mg, 0.0444 mmol) in $CHCl_3$ (1 ml) was added a pH 7 phosphate buffer (5 ml) and an aqueous solution of KI (24 mg, 0.144 mmol dissolved in 0.3 ml of H_2O) at 0°C. The solution was stirred for 10 min followed by the addition of **14** (20 mg, 0.044 mmol) in a mixture of $CHCl_3$ (5 ml) and DMF (0.3 ml). After stirring at 0°C for 15 min, the mixture was diluted with CH_2Cl_2 (40 ml). The organic layer was separated and washed with an aqueous $Na_2S_2O_5$ solution (5%, 5 ml), H_2O (10 ml), dried ($MgSO_4$), filtered and concentrated on a rotary evaporator. The residue was triturated in hot CH_3OH, cooled to RT, and filtered to yield **3a** (23 mg, 74%) as a white powder. MS m/e 705 $[M+H]^+$.

^{125}I labeled 1-(4-Hydroxy-3-iodobenzyl)-3-(1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-2,3-dihydro-1H-benzoimidazol-2-ylmethyl)-1,3-dihydrobenzo-imidazol-2-one, **2** *and ^{125}I labeled 1-(4-Hydroxy-3-iodobenzyl)-3-(1-[2-(3-methyl-3H-diazirin-3-yl)-ethyl]-2,3-dihydro-1H-benzoimidazol-2-ylmethyl)-1,3-dihydrobenzo-imidazol-2-one*, **3**: *The following procedure was conducted under red light*. Into a 5 ml conical reaction vial was added 15 µl of a solution containing 1 mg/ml of **14** dissolved in 40% DMF/EtOH, 10 µl of a solution of 2 mg/ml of iodogen dissolved in EtOH, 15 µl of $NaHPO_4$ buffer 0.05 M, pH 8.0) and 10–15 µl of $Na^{125}I$. The solution was then allowed to stir at room temperature for 10 min

prior to quenching the reaction with 15 µl of a solution of $Na_2S_2O_5$ (9 mg/ml). The sample was purified by HPLC (Method C). Each product was then concentrated in a Savant Speedvac and redissolved in EtOH. The specific activity of **2** and **3** were assumed to be 2100 and 4200 Ci/mmol, respectively, based upon the specific activity of the $Na^{125}I$ used in these experiments.

Radio-iodination experiments ($n=4$) were completed with routine radiochemical yields of **2** + **3** ranging from 45 to 60%. In addition, relative yields of **2** and **3** were comparable with **3** usually being prepared in slightly higher yields. In a typical radio-iodination experiment starting with 2.7 mCi of $Na^{125}I$, we obtained 0.7 mCi of **2** and 0.8 mCi of **3** following HPLC purification.

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