PHOTOCHEMICALLY ACTIVATED ANTIVIRAL HALOGENATED 1,8-NAPHTHALIMIDES: SYNTHESIS OF *N,N'-BIS*-{2-[(5-BROMO-2-[1-^{14}C]HEXYL-1*H*-BENZ[*DE*]ISOQUINOLIN-1,3(2*H*)-DION-6-YL)AMINO]ETHYL}HEXANEDIAMIDE

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SUMMARY

The synthesis of *N,N'-bis*-{2-[(5-bromo-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dion-6-yl)amino]ethyl}hexanediamide from 1-[1-^{14}C]-hexylamine and 4-chloro-1,8-naphthalic anhydride is described. The anhydride is first converted to the 4-chloro-*N*-[1-^{14}C]hexyl-1,8-naphthalimide (**2**) by condensation with 1-[1-^{14}C]-hexylamine, and the chlorine is then displaced with ethylenediamine to give the 4-(2-aminoethylamino-*N*-[1-^{14}C]hexyl-1,8-naphthalimide (**3**). Coupling of this monomeric naphthalimide with adipoyl chloride affords the dimeric naphthalimide (**4**) which is brominated regiospecifically with elemental bromine in carbon tetrachloride to afford the title compound (**5**).

KEY WORDS: Antiviral; Photochemically activatable; 4-Alkylamino-3-bromo-1,8-naphthalimides; *N,N'-bis*-{2-[5-bromo-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dion-6-yl)amino]ethyl}-hexanediamide.

INTRODUCTION

Phototargeted chemotherapy is an area of intense interest and study. Thus, visible light has been used to effect cell kill in tumors or in disorders such as psoriasis by using a membrane-bound dye to mediate the photochemical inactivation process (1). Where the photosensitizer is a dye, it is

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generally accepted that cell death is due to the action of singlet oxygen or other peroxidic photoproducts generated within the cell membrane leading to oxidative degradation (2).

Recently, it has been discovered that compounds based on the 3-bromo-4-alkylamino-1,8-naphthalimide skeleton constitute a new class of photochemically activatable antiviral compounds that are characterized by being effective cross-linkers of membrane proteins in the absence of molecular oxygen. At sub-micromolar concentrations, the dimeric 3-bromo-4-alkylamino-*N*-alkyl-1,8-naphthalimides inactivate enveloped viruses and cells (including HIV-1) upon photochemical activation with blue light in the 420-435 nm range, and they inhibit syncytium formation by HIV-1–infected cells under similar circumstances (3). Moreover, at the concentrations effective in neutralizing HIV-1 infectivity, these compounds retain low toxicity towards normal cells, and extremely low toxicity in the absence of light. The activation of the dye has been carried out both *in situ*, after incubation of the virus or cells with dye and washing to remove unbound dye, and by preactivation – photochemical activation of the dye itself prior to incubation with viruses or cells (4); interestingly, the monomeric 3-bromo-4-alkylamino-*N*-alkyl-1,8-naphthalimides are much more amenable to preactivation than the dimeric compounds. The compound formed by irradiation and bleaching of the monomeric dye prior to incubation with the retrovirus or cells inhibits syncytium formation by HIV-1–infected cells, but does not reduce the infectivity of cell-free virus. The dimeric naphthalimides that function as photochemically activatable antiviral agents have been shown to be effective cross-linking agents for collagen in a variety of tissues, including dura mater (5).

In order to pursue studies of the toxicity and mechanism of action of these compounds, the appropriate ^{14}C labeled materials were required, and the synthesis of *N,N'-bis*-{2-[(5-bromo-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dion-6-yl)amino]ethyl}hexanediamide, a representative of the antiviral halogenated naphthalimides of this class, was undertaken.

EXPERIMENTAL

General

Solvents and reagents were used as obtained form Aldrich Chemical Company unless otherwise specified. Melting points were determined using an electrically heated hot-stage microscope, and are uncorrected. Radioassays were carried out by liquid scintillation using a Packard 1500 Tri Carb liquid scintillation counter. 1-[1-^{14}C]hexylamine was prepared in 81% yield by standard methods from potassium [^{14}C]cyanide by sequential treatment with excess 1-bromopentane in refluxing ethanol and lithium aluminum hydride in ether.

6-Chloro-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dione (2).

To a stirred mixture of freshly recrystallized (absolute ethanol) 4-chloro-1,8-naphthalic anhydride (371 mg, 1.6 mmol) and xylene (5 mL) was added 1-[1-^{14}C]hexylamine (101.2 mg, 1.5 mmol, 10 mCi) in xylene (2 mL). Heating under reflux (16 h) followed by cooling gave a yellow precipitate which was recrystallized from ethanol. Subjection of this material to column chromatography (silica gel, hexane/ethyl acetate (8:1)) afforded 6-chloro-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dione (300 mg, 81%, 7.7 mCi, S.A. 9.6 mCi/mmol) as a pale yellow solid, m. 67-68°C.

6-(2-Aminoethyl)amino-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dione (3).

6-Chloro-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dione (300 mg, 0.95 mmol) was dissolved in anhydrous ethylenediamine (5 mL) and the reaction mixture was heated under reflux with stirring for 16 h. Solvent removal gave the crude product as a red-brown solid. Recrystallization from methanol afforded 6-(2-aminoethyl)amino-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dione as an orange solid (318 mg, 98.7%, 7.6 mCi, S.A. 8.3 mCi/mmol). This compound is freely soluble in dichloromethane, but on storage undergoes radiolysis to afford material of partial solubility.

***N,N'-bis*-{2-[(2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dion-6-yl)amino]-ethyl}hexanediamide (4).**

To a solution of 6-(2-aminoethyl)amino-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dione (65 mg, 0.19 mmol) and pyridine (60 mg, 0.76 mmol) in dichloromethane (150 mL) was added a solution of adipoyl chloride (18 mg, 0.09 mmol) in dichloromethane (1 mL) over 5 minutes by dropwise addition. The solution was allowed to stir at ambient temperature for 16 h, poured into 10% HCl (100 mL) and the organic layer was separated, washed with water, and dried (Na_2SO_4). Solvent removal gave crude *N,N'-bis*-{2-[(2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dion-6-yl)-amino]ethyl}hexanediamide (49 mg, 69%, 1.1 mCi) as an orange-red solid that was used without further purification.

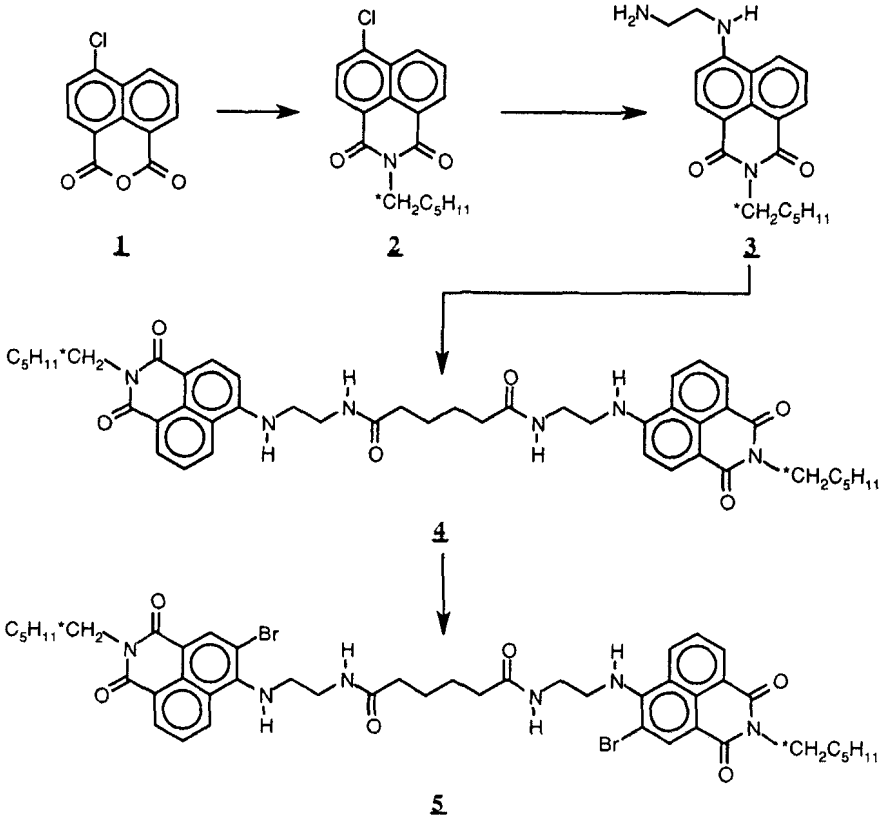
***N,N'-bis*-{2-[(5-bromo-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dion-6-yl)-amino]ethyl}hexanediamide (5).**

To a solution of the dimeric naphthalimide **4** (145 mg, 0.18 mmol) in carbon tetrachloride (300 mL) was added a solution of bromine (58 mg, 0.36 mmol) in the same solvent (2 mL), dropwise, over

5 minutes. The solution was allowed to stir at ambient temperature for 12 h after which time t.l.c. (eluant: 10% ethanol in acetic acid) indicated that the starting material had been consumed. Solvent removal under reduced pressure afforded the crude product as a brick-red solid which was recrystallized from 1-butanol to give *N,N'-bis*-{2-[(5-bromo-2-[1-^{14}C]hexyl-1*H*-benz[*de*]isoquinolin-1,3(2*H*)-dion-6-yl)amino]ethyl}hexanediamide (109 mg, 75%, 1.85 mCi, S.A. 16 mCi/mmol) as orange-red crystals exhibiting hyperchromism in the uv-visible spectrum.

RESULTS AND DISCUSSION

The choice of the radiolabeled compound for this study was predicated on two major concerns: the need for the radiolabel to be incorporated at a relatively chemically unreactive site in the molecule, and the need for the synthesis to be simply adapted from the synthesis of the unlabeled material from readily available starting materials. Based on these considerations, the location for the radiolabel was chosen as the α carbon of the *N*-alkyl side chain of the naphthalimide nucleus using a synthetic



[* = position or radiolabeled atom]

pathway adapted from the published synthesis (3). The imide ring of the naphthalimide, which is extremely resistant to hydrolytic cleavage once formed, was an obvious position for the radiolabel, either in the ring itself or in the *N*-alkyl side chain. Since neither the naphthalic anhydrides nor the naphthalimides are available in radiolabeled form, the simplest source of carbon 14 was 1-[1-^{14}C]hexylamine.

Thus, the reaction between 4-chloro-1,8-naphthalic anhydride (**1**) and 1-[1-^{14}C]hexylamine initially affords an off-white, sparingly soluble acid amide which undergoes cyclization in refluxing xylene to afford the condensation product, the 4-chloro-*N*-[1-^{14}C]hexyl-1,8-naphthalimide (**2**). Despite being an aromatic halide, the chlorine atom in the imide (**2**) is activated towards nucleophilic substitution by both carbonyl groups of the imide ring. When the imide was subjected to nucleophilic displacement with excess anhydrous ethylenediamine, the corresponding 4-alkylamino-*N*-alkyl-1,8-naphthalimide (**3**) was obtained. The imide (**3**) was freely soluble in dichloromethane when freshly prepared, but it underwent radiolysis on storage to afford an insoluble material, so it was immediately acylated with 0.5 equivalents of adipoyl chloride to afford the dimeric amide (**4**) as an orange-red solid. Bromination of the dimeric amide (**4**) was regiospecific as reported (3a), and it proceeded without incident as in the published procedure to give the dimeric brominated naphthalimide (**5**).

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