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Synthesis and radioiodination of some daunorubicin and doxorubicin derivatives

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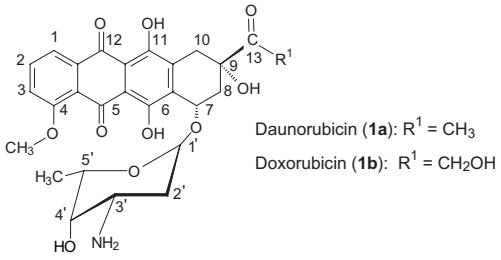
Abstract—Daunorubicin and doxorubicin are efficient agents for cancer treatment. Their clinical efficacy is, however, hampered by their indiscriminant toxicity. This problem may be circumvented by encapsulating the drugs in liposomes and selectively targeting the tumor cells using tumor targeting agents. Furthermore, the antitumor effect could be enhanced by attaching the Auger electron emitter, ^{125}I, to daunorubicin and doxorubicin derivatives. In this context a number of ester, amide, and amine derivatives of daunorubicin and doxorubicin were synthesized. Benzoic acid ester derivatives of daunorubicin were synthesized by nucleophilic esterification of the 14-bromodaunorubicin with the potassium salt of the corresponding benzoic acid, resulting in good yields. Nicotinic acids and benzoic acids, activated with a succinimidyl group, were coupled to the amino group of daunorubicin to give the corresponding amide derivatives. Amine derivatives were obtained by the reductive amination of aromatic aldehydes with daunorubicin hydrochloride. The stannylated ester and amide derivatives were used as precursors for radioiodination. Radiolabeling with ^{125}I was performed using chloramine-T as an oxidant. The optimized labeling resulted in high radiolabeling yields (85–95%) of the radioiodinated daunorubicin and doxorubicin derivatives. Radioiodination of the amines was conducted at the *ortho* position of the activated phenyl rings providing moderate radiochemical yields (55–75%).

Keywords: Daunorubicin; Doxorubicin; Radioiodination; ^{125}I; Chloramine-T

1. Introduction

Daunorubicin (**1a**) and doxorubicin (**1b**) are the most extensively used anthracycline antitumor antibiotics. Daunorubicin was the first antibiotic of this class to show activity against acute leukemia in humans. Later studies have shown that it also has activity against solid tumors.[1,2] The substitution of one hydrogen atom in the daunorubicin acetyl side chain with an hydroxyl group gives doxorubicin. Doxorubicin exhibits antitumor activity against solid tumors, such as breast and lung cancers, and is generally known to be more potent than daunorubicin. Both compounds are known for their effective intercalation to DNA.



The generation of free radicals following microsomal or chemical activation of the quinone moiety of the anthracyclines is believed to contribute to either their cytotoxicity or cardiotoxicity.[3] Many derivatives of these compounds have therefore been synthesized to improve their cytotoxicity and decrease their cardiotoxicity.[4–10] The mechanism of the antibiotic action of these molecules is related to their ability to intercalate between adjacent

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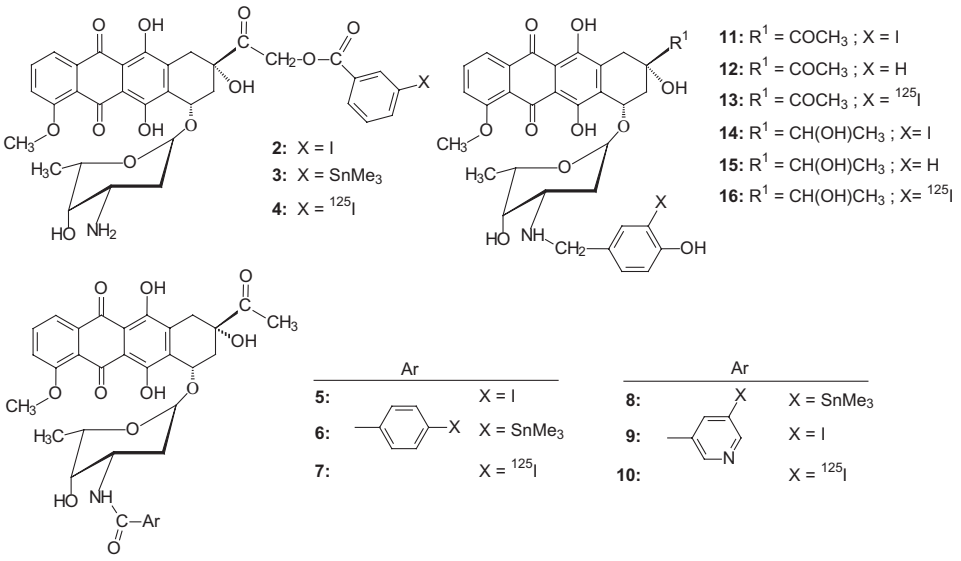


base pairs causing topoisomerase II inhibition, which leads to the production of hydroxyl free radicals that are toxic to both normal and tumor cells.[11] The problems that limit the clinical efficacy of these agents are their cardiotoxicity and multidrug resistance.[12] One approach that possibly improves their therapeutic ability is targeting these molecules specifically to the cancer cells.[13]

In this report, we present the synthesis of some daunorubicin and doxorubicin derivatives as potential candidates for targeted nuclide therapy. The compounds are esters **2**–**4**, amides **5**–**10**, and amines **11**–**16**.

The precursor compounds are labeled with ^{125}I and will be loaded into liposomes. We plan to investigate their efficacy using a two-step targeting technique.[14] In the first step, stabilized liposomes conjugated with a targeting agent will be actively loaded with the labeled compounds and injected into blood circulation. After binding to cancer cells, the loaded liposomes will be internalized, and labeled daunorubicin and doxorubicin derivatives will be released into the cytoplasm. In the second step, the compounds, with the help of the DNA intercalating part, are supposed to bind to the DNA of the tumor cell. The ^{125}I nuclide emits Auger electrons that travel short distances, but are highly cytotoxic when emitted in close vicinity to DNA. In preliminary experiments, two of these compounds, **13** and **16**, were found to be taken up by a permeabilized cell line and transit into the cell nucleus. The biological experiments will be presented elsewhere.

2. Results and discussion

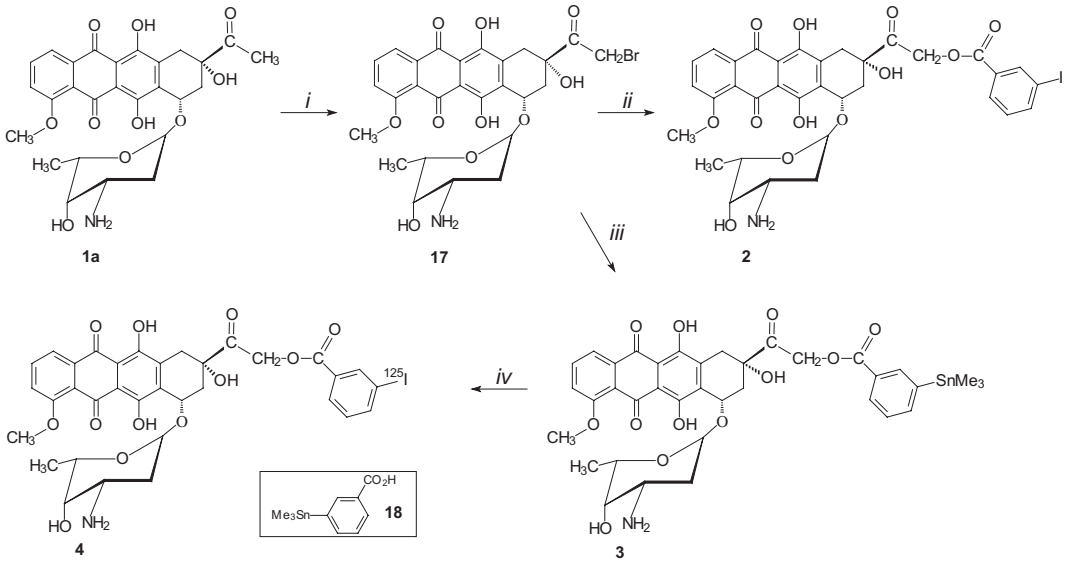
2.1. Synthesis

2.1.1. Synthesis of esters 2–4. Synthesis of compounds **2**–**4** was performed as shown in Scheme 1. Bromination of the hydrochloride of daunorubicin using bromine in the presence of trimethylorthoformate, as described in the literature,[15] gave 14-bromo-13-dimethyl acetal of daunorubicin, which upon being stirred in acetone provided 14-bromodaunorubicin (**17**), in high yield. The Stille coupling reaction of 3-iodobenzoic acid and hexamethylditin using a palladium(II) catalyst provided an 88% conversion to the corresponding stannyl compound **18**, as described in the literature.[16]

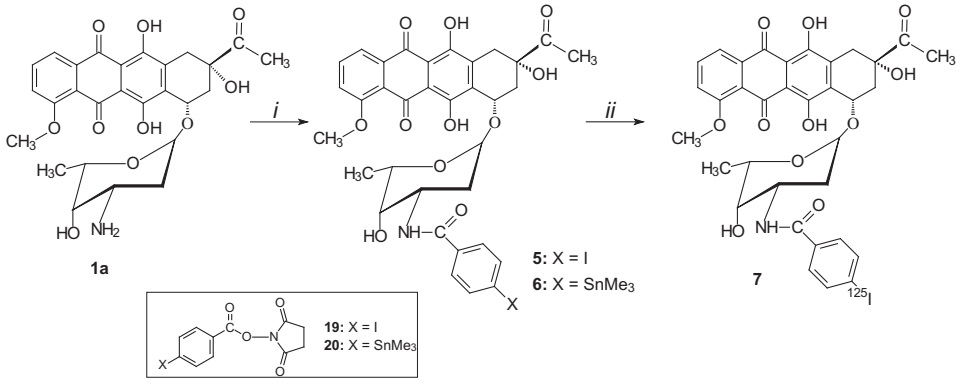
The reaction of 3-iodobenzoic acid with the bromo compound **17** and K_2CO_3 at room temperature produced a good (73%) yield of the ester, **2**. Similarly, synthesis of compound **3** was carried out with the stannylated compound **18**, providing a high yield of the target. Both esters were purified by flash chromatography. The purity and identity of the compounds were confirmed by LC/MS and NMR.

2.1.2. Synthesis of amides 5–10. The amides **5**–**7** were synthesized as outlined in Scheme 2. 4-Iodobenzoic acid and 4-(trimethylstannyl)benzoic acid were reacted with di-(*N*-succinimidyl)carbonate in pyridine to yield the corresponding *N*-succinimidyl-derivatives (**19** and **20**, respectively), as described previously.[16] These activated compounds, **19** and **20**, were reacted with daunorubicin hydrochloride in the presence of a 5-fold molar excess of triethylamine in DMF to give 74% and 86% yields of 3′-*N*-4-iodo-benzoyldaunorubicin, **5**, and 3′-*N*-4-trimethylstannylbenzoyldaunorubicin, **6**, respectively.

The syntheses of the amides **8**–**10** are outlined in Scheme 3. The previously known compound *N*-succinimidyl-5-bromo-nicotinic acid (**21**),[17] was synthesized by the same procedure as was used for **19**, except that 5-bromonicotinic acid was used as the starting material. The activated stannylated ester **22**[17] was obtained in a low yield from **21** using the same method as described for the preparation of **20**. *N*-Succinimidyl 5-(trimethylstannyl)nico-



Scheme 1. (i) (a) $CH(OMe)_3$, MeOH/dioxane (1:2), rt; (b) $Br_2/CHCl_3$, 30 °C; (c) acetone, rt; (ii) 3-iodobenzoic acid, K_2CO_3, rt, and (iii) compound **18**, K_2CO_3, rt; (iv) $Na^{125}I$, 3% AcOH/MeOH, CAT, $Na_2S_2O_5$, NaI.



Scheme 2. (i) For **5**: **19**, triethylamine, rt, 1.5 h; for **6**: **20**, triethylamine, rt, 1.5 h, and (ii) $Na^{125}I$, 1% AcOH/MeOH, CAT, $Na_2S_2O_5$.

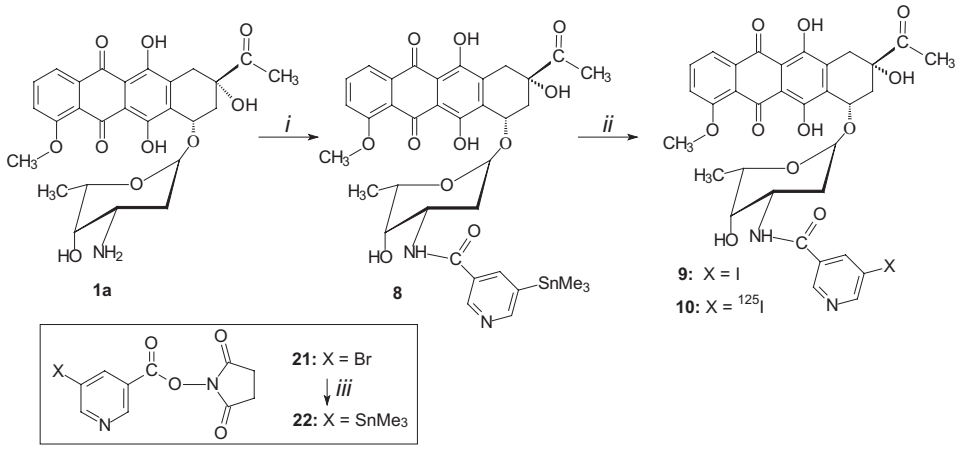
tinic acid (**22**) was reacted with daunorubicin hydrochloride using the same procedure as was used for the preparation of **6**. The crude product was purified by column chromatography to give a 72% yield of compound **8**. The stannylated precursor was iodinated using NaI in 1% acetic acid/methanol, and chloramine-T (CAT) was used as an oxidant to produce a 77% yield of **9**.

2.1.3. Synthesis of amines 11–16. The amino-benzyl derivatives were synthesized as outlined in Scheme 4. Compound **12** was prepared by the reductive amination of 4-hydroxybenzaldehyde with daunorubicin hydrochloride using sodium cyanoborohydride. The reaction was very slow and thus required more than 1 equiv of the reagents. After 3 days of reaction at room temperature, the starting daunorubicin hydrochloride was totally consumed, according to thin-layer chromatography (TLC). The mixture was purified by chromatography and analyzed by NMR and LC/MS. Two major fractions were isolated; these were found to be compounds **12** and **15** (as a mixture of two diastereomers). Compound **15**, which was formed from reduction of the carbonyl functional group at position 13 due to the excess sodium cyanoborohydride, was the major product. 4-Hydroxybenzaldehyde was iodinated with I_2/Ag_2SO_4. The reaction afforded a moderate yield of the monoiodo derivative, **23**.[18] Compounds **11** and **14** (as a mixture of two diastereomers), were prepared from 4-hydroxy-3-iodobenzaldehyde **23** and daunorubicin hydrochloride using a procedure similar to that used for the synthesis of **12** and **15**. Similarly, compound **14** was the major product.

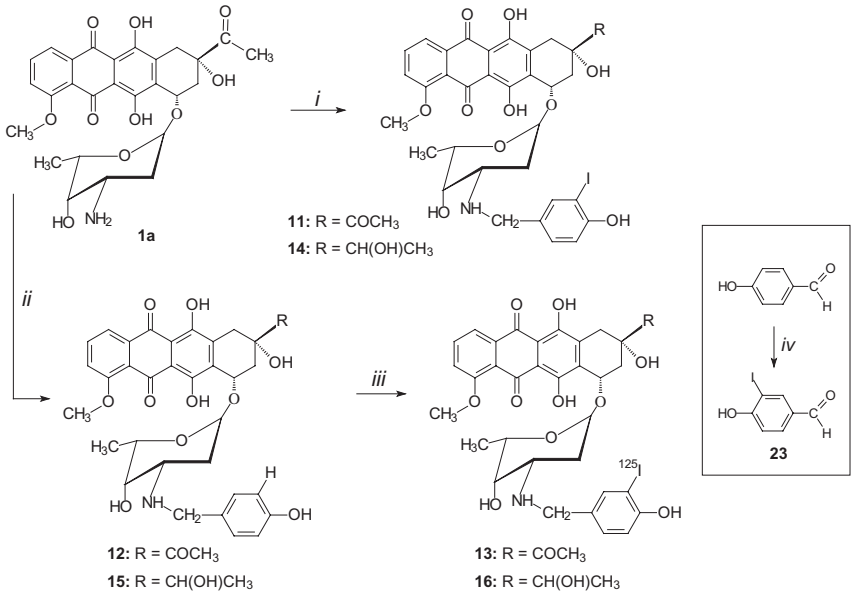
3. Radiolabeling

3.1. Radioiodination

Compounds **3**, **6**, **8**, **12**, and **15** were used as precursors for the radioiodination. All the compounds were labeled



Scheme 3. (i) Compound **22**, triethylamine, rt, 1.5 h; (ii) for **9**: NaI, chloramine-T, $Na_2S_2O_5$, rt, 13 min; for **10**: $Na^{125}I$, 1% AcOH/MeOH, CAT, $Na_2S_2O_5$, NaI; (iii) $Pd(PPh_3)_2Cl_2$, Sn_2Me_6, 1,4-dioxane.



Scheme 4. (i) Aldehyde **23**, MeCN–H_2O (2:1), 1 M solution $NaBH_3CN$/THF, rt, and (ii) 4-hydroxybenzaldehyde, MeCN–H_2O (2:1), 1 M solution $NaBH_3CN$/THF, rt; (iii) $Na^{125}I$, MeOH, CAT, $Na_2S_2O_5$, NaI; (iv) I_2, Ag_2SO_4, CH_2Cl_2, rt.

with ^{125}I, and chloramine-T was used as an oxidant. The labeling was performed with no carrier added. The influence of some parameters, such as amount of oxidant, amount of substrate, and reaction time on labeling efficiency was investigated. The acidity of the reaction media was also found to have a great effect in the cases of some of the derivatives. The radioiodination yields are shown in Table 1.

Figure 1a displays a typical radio-TLC chromatogram of [^{125}I] iodide radioiodination of the model compound, **6**, using the final optimized reaction conditions. The optimized conditions were found to be 80 μg of compound **6** in 40 μL of 1% acetic acid in methanol solution, 5 μL [^{125}I]iodide solution (3.7 GBq/mL), and 10 μL chloramine-T (CAT) solution (8 mg/mL in methanol).

Table 1. Radioiodination yield of daunorubicin/doxorubicin derivatives

Entry	Radioiodinated compounds	Time (min)	Yield (%) ($n = 2$)
1	**4**	5	85 ± 0.2
2	**7**	5	95 ± 0.2
3	**10**	5	91 ± 0.8
4	**13**	5	53 ± 1.2
5	**16**	5	72 ± 1.6

All radio-TLC analyses were performed with NaI as a carrier, which was added after labeling to stabilize the radioiodide by protecting it from oxidation during elution of the TLC plates. From the TLC analysis, peak

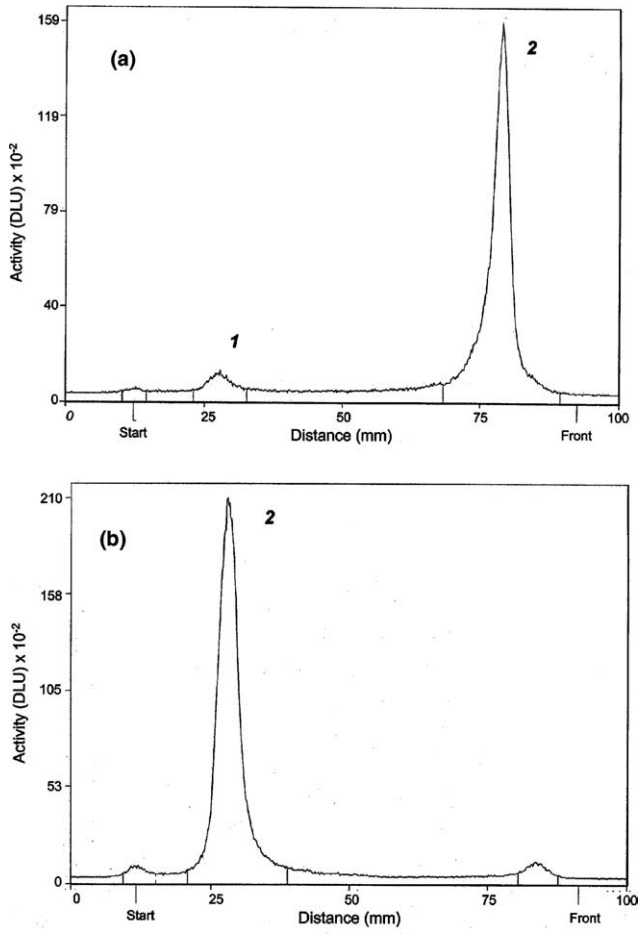


Figure 1. (a) Typical TLC chromatogram of compound **7** using the general labeling procedure. Peak 1 corresponds to ^{125}I stabilized with carrier NaI ($R_f = 0.3$) against oxidation, and peak 2 has the same retention factor ($R_f = 0.8$) as the iodinated nonradioactive reference **5**, thus corresponding to radiolabeled compound **7**; (b) typical TLC radiochromatogram of a blank experiment.

1 ($R_f = 0.3$) was found to be Na^{125}I. Peak 2 ($R_f = 0.8$) has the same retention factor as the nonradioactive iodinated reference compound **5** and is thus associated with the labeled compound **7**. A blank experiment (Fig. 1b) was performed using exactly the same conditions, except that a neat methanol solution was used instead of the substrate daunorubicin/doxorubicin derivatives.

The dependence of radiochemical yield on various reaction conditions was investigated using compound **15** as shown in Figure 2a–c. As can be seen from Figure 2a, radiochemical yield is strongly dependent on reaction time, the yield reaching a plateau after an incubation time of 4 min. Further increase in reaction time was found to have no impact on the labeling yield.

The effect of oxidant concentration was examined by varying the concentration of CAT. The best result was obtained with 80 μg (0.35 μmol) CAT, which is a 5.6:1 molar ratio of CAT to the precursor. Reducing the amount of CAT to less than 40 μg resulted in a sharp decrease in yield. Increasing the amount of CAT beyond 80 μg had no effect on the labeling yield (Fig. 2b).

The dependence of radiochemical yield on the amount of substrate is depicted in Figure 2c. To investigate the influence of substrate (compound **15**) concentration on radiolabeling yield, the reaction was carried out at different concentrations in the 1–4 mg/mL range. The radiolabeling yield increased with increasing amounts of substrate. The best yield (72% for compound **16**) was obtained with a reaction mixture containing at least 80 μg of the substrate.

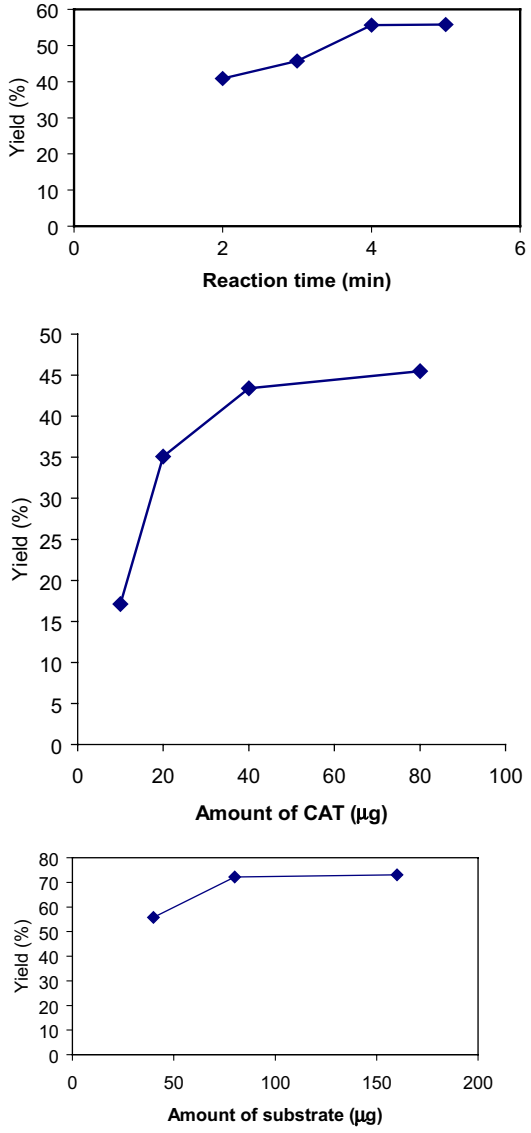


Figure 2. (a) Influence of reaction time on radioiodination yield. Other conditions were: amount of substrate **15**, 40 μg; amount of CAT, 80 μg; and total volume, 55 μL; (b) influence of CAT on radioiodination yield. Other conditions were: amount of substrate **15**, 40 μg; reaction time, 5 min; and total volume, 55 μL; (c) influence of substrate **15** on the radioiodination yield. Other conditions were: amount of CAT, 80 μg; reaction time, 5 min; and total volume, 55 μL. Data in all three figures represent average values from two experiments.

Further improvement of the labeling of the stannylated precursors was associated with acidification of the reaction media using 1% acetic acid in methanol. The reaction achieved high to excellent labeling yield of the corresponding [^{125}I]-iodinated products. The other compounds were also subjected to the same reaction conditions to determine the effect of acidity on radiochemical yield. However the addition of acid was found to have very little effect on the labeling of the non-stannylated precursors.

4. Experimental

4.1. General methods

4.1.1. Synthesis. ^{1}H and ^{13}C spectra were recorded in $CDCl_3$ (7.26 ppm ^{1}H, 77.0 ppm ^{13}C) or CD_3OD (3.35 ppm ^{1}H, 49.0 ppm ^{13}C) on a Varian Unity 400 spectrometer operating at 400 and 100.6 MHz, respectively. Merck silica gel 60 (230–400 mesh) was used for column chromatography. TLC was performed using Merck Silica 60 F_{254} gel. All chemicals were of p.a. grade and used as supplied by Aldrich©. DMF, CH_2Cl_2, 1,4-dioxane, $CHCl_3$, and MeOH were dried using standard methods.[19] An AQA mass spectrometer and an electrospray positive ionization method in MeOH solution was used for LC/MS analysis. HRMS was conducted at the Organisch Chemisches Institut der Universitaet Muenster, Germany. The acronyms 'benz' and 'pyr' are used for benzene and pyridine rings, respectively.

4.1.2. Radiolabeling and analysis. [^{125}I] iodide (3.7 GBq/mL, specific activity 644 MBq/μg) was obtained from Amersham Pharmacia Biotech UK Limited. MeOH (HPLC grade) was used as supplied. High quality ELGA water (resistance higher than 18 MΩ/cm^3) was used in preparing aqueous solutions. *N*-Chloro-*p*-toluenesulfonamide sodium salt (chloramine-T, CAT) and sodium metabisulfite, $Na_2S_2O_5$ were purchased from Sigma. Sodium iodide, NaI, pro analysis was obtained from Merck. The solutions of the daunorubicin/doxorubicin derivatives, CAT (1, 2, 4, and 8 mg/mL solutions in MeOH), $Na_2S_2O_5$ (8 and 16 mg/mL solutions in H_2O), and NaI (10 mg/mL solution in H_2O) were always prepared fresh.

Silica gel 60 F_{254} thin-layer chromatography plates (E. Merck, Darmstadt, Germany) were used for analysis. The reaction mixture (1–2 μL) was applied to a TLC plate. A mixture of MeOH (15 mL), ether (5 mL) and acetic acid (40 μL) was used as eluent for compounds **13** and **16**; 4:1:0.002, CH_2Cl_2–MeOH–HCO_2H was used for compound **4**, and a mixture of 5:1, Et_2O–MeOH for compounds **7** and **10**. The R_f values for the nonradiolabeled iododaunorubicin/doxorubicin derivatives were used for comparison and they are the same as those of their radiolabeled analogues. The positions of standard chromatography spots were determined visually either using UV or with the naked eye. The distribution of radioactivity along the TLC strips (100 × 50 mm; elution path, 80 mm) was measured on the Cyclone™ storage phosphor system (Packard Instruments Company Inc., Downers Grove, US) and analyzed using the OPTIQUANT™ image analysis software.

4.2. Adriamycin 14-*O*-3-iodobenzoate (2)

14-Bromodaunorubicin **17** (15 mg, 0.023 mmol), and 3-iodobenzoic acid (11.6 mg, 0.047 mmol) were dissolved in acetone under an inert atmosphere. Potassium carbonate (9.5 mg, 0.069 mmol) was added and the reaction mixture was stirred at rt for 12 h. The solvent was evaporated and the crude product was purified by column chromatography (4:1, CH_2Cl_2–MeOH) to give compound **2** as a red powder (13 mg, 73%). The purity of the compound was checked by TLC and LC/MS. The compound was converted to its hydrochloride salt by adding HCl in ether to a THF solution of the compound and then stirring at −20 °C for 2 h.^{1}H NMR (CD_3OD): δ 8.39 (s, 1H, Ar), 8.02 (d, 1H, J = 8.1 Hz, H-3), 7.96 (d, 1H, J = 8.6 Hz, Ar), 7.87 (d, 1H, J = 8.1 Hz, H-1), 7.72 (t, 1H, J = 8.1 Hz, H-2), 7.35 (d, 1H, J = 8.6 Hz, Ar), 7.17 (t, 1H, J = 8.6 Hz, Ar), 5.46 (s, 1H, H-1′), 5.3–5.25 (m, 2H, H-14a, H-14b), 5.19 (s, 1H, H-7), 4.15 (q, 1H, J = 6.5 Hz, H-5′), 4.01 (s, 3H, OCH_3), 3.74 (m, 2H, H-3′, H-4′), 3.24 (d, 1H, J = 20.0 Hz, H-10), 3.00 (d, 1H, J = 20.0 Hz, H-10), 2.43 (m, 1H, H-8), 2.13 (m, 1H, H-8), 1.97 (m, 1H, H-2′), 1.82 (m, 1H, H-2′), 1.29 (d, 3H, J = 6.5 Hz, CH_3); ^{13}C NMR (CD_3OD): δ 216.9, 178.2, 164.7, 164.7, 161.2, 142.3, 141.0, 138.4, 131.6, 130.4, 129.6, 128.8, 121.8, 119.3, 113.3, 100.1, 93.4, 76.4, 70.3, 67.2, 66.9, 55.9, 34.5, 29.6, 8.8, 15.9. ESMS: *m*/*z* calcd for [$C_{34}H_{32}INO_{12} + H^+$]: 774.1. Found: *m*/*z* 774.1.

4.3. Adriamycin 14-*O*-(3-trimethylstannyl)benzoate (3)

14-Bromodaunorubicin **17** (30.0 mg, 0.047 mmol), and 3-trimethylstannylbenzoic acid (26.6 mg, 0.093 mmol) were dissolved in acetone under an inert atmosphere. Potassium carbonate (19.3 mg, 0.14 mmol) was added and the reaction mixture was stirred at rt for 2 h. The solvent was evaporated and the residue was purified by column chromatography (4:1, CH_2Cl_2–MeOH), providing **3** (35.0 mg, 91%). ^{1}H NMR (CD_3OD): δ 8.20 (s with tin satellites, $J_{H,Sn}$ = 28 Hz, 1H, Ar), 8.01 (d, 1H, J = 7.7 Hz, H-3), 7.76–7.66 (m, 3H, H-1, H-2, Ar), 7.46 (t, 1H, J = 8.3 Hz, Ar), 7.41 (t, 1H, J = 8.3 Hz, Ar), 5.56 (d, 1H, J = 18.0 Hz, H-14a), 5.43 (s, 1H, H-1′), 5.34 (s, 1H, H-14a), 4.98 (s, 1H, H-7), 4.35 (q, 1H, J = 6.8 Hz, H-5′), 3.94 (s, 3H, OCH_3), 3.70 (br s, 1H,

H-4′), 3.55 (m, 1H, H-3′), 3.04 (d, 1H, $J = 20.1$ Hz, H-10), 2.72 (d, 1H, $J = 20.1$ Hz, H-10), 2.51 (m, 1H, H-8), 2.12–1.98 (m, 2H, H-8, H-2′), 1.91 (m, 1H, H-2′), 1.34 (d, 3H, $J = 6.8$ Hz, CH_3), 0.32 (s with tin satellites, $J_{H,Sn} = 28$ Hz, 9H, $Sn(CH_3)_3$); ^{13}C NMR (CD_3OD): δ 208.6, 187.7, 187.4, 167.9, 162.3, 157.2, 155.9, 144.2, 141.9, 137.7, 137.2, 136.0, 135.4, 135.1, 130.6, 130.1, 129.2, 121.1, 120.3, 112.2, 112.0, 101.3, 77.5, 71.4, 68.3, 68.1, 57.1, 37.0, 33.4, 29.9, 17.1, 9.8. HRMS: *m/z* calcd for $[C_{37}H_{41}NO_{12}Sn + Na^+]$: 834.1140. Found: *m/z* 834.1151.

4.4. 3′-*N*-4-Iodo-benzoyldaunorubicin (5)

A 10 mg/mL solution of **19** in dry dimethylformamide (2 mL) was added in a 1:1 molar ratio to daunorubicin hydrochloride (20 mg/mL in DMF). A 5-fold molar excess of triethylamine (17.94 mg, 0.18 mmol) was injected into the reaction mixture; which was then stirred at rt under an argon atmosphere for 1.5 h. The course of the reaction was followed by reversed phase-HPLC, using a gradient of 80% water–20% acetonitrile (ACN), changing to 100% ACN over 10 min, then held for 15 min (flow rate = 1 mL/min, UV monitoring at 254 nm). After 1.5 h, the reaction was complete. The solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography using EtOAc–pentane, 70:30 as a mobile phase to give **5** (19.9 mg, 74%) as a red solid; ^1H NMR ($CDCl_3$): δ 13.99, 13.26 (d, 2H, 6,11-OH), 8.03 (dd, 1H, $J = 7.8$, 1.1 Hz, 1-H), 7.77 (dd, 1H, $J = 7.8$, 8.5 Hz, 2-H), 7.72 (m, 2H, AA′ part of AA′XX′), 7.37 (dd, 1H, $J = 8.5$, 1.1 Hz, 3-H), 7.42 (m, 2H, XX′ part of AA′XX′), 5.52 (m, 1H, 1′-H), 5.27 (m, 1H, 7-H), 4.44 (s, 1H, 9-OH), 4.37 (m, 1H, 3′-H), 4.29 (m, 1H, 5′-H), 4.05 (s, 3H, 4-OCH_3), 3.73 (m, 1H, 4′-H), 3.24 (dd, 1H, $J = 18.9$, 1.9 Hz, 10-H_{eq}), 2.92 (d, 1H, $J = 18.8$ Hz, 10-H_{ax}), 2.42 (s, 3H, 14-CH_3), 2.34 (m, 1H, 4′-OH), 2.16–2.14 (m, 2H, 8-H_{eq}, 8H_{ax}), 2.00 (m, 1H, m, 2′-H_{eq}), 1.85 (m, 1H, 2′-H_{ax}), 1.31 (d, 3H, $J = 6.6$ Hz, 5′-CH_3); ^{13}C NMR ($CDCl_3$): δ 212.3, 187.3, 186.9, 166.0, 161.3, 156.6, 156.1, 138.0, 135.9, 135.8, 134.7, 134.2, 133.9, 128.8, 121.2, 120.1, 118.6, 111.8, 111.6, 100.9, 98.7, 70.4, 69.9, 67.3, 56.9, 46.1, 46.0, 35.4, 33.7, 30.3, 25.0, 17.0. HRMS: *m/z* calcd for $[C_{34}H_{32}INO_{11} + Na^+]$: 780.0918, $[C_{34}H_{32}INO_{11} + K^+]$: 796.0657. Found: *m/z* 780.0875, 796.0640, respectively.

4.5. 3′-*N*-4-Trimethylstannylbenzoyldaunorubicin (6)

The synthesis of **6** was carried out as outlined for **5** except that *N*-succinimidyl-4-(trimethylstannyl)benzoic acid (**20**) was used as the starting material. The course of the reaction was followed by TLC (EtOAc–pentane, 70:30). The solvent was evaporated under reduced pressure to dryness at rt. The crude product was purified by flash chromatography using EtOAc–pentane, 70:30 as a mobile phase to give **6** (36.2 mg, 86%) as a red solid. ^1H NMR (CD_3OD): δ 7.90 (dd, 1H, $J = 7.7$, 1.1 Hz, 1-H), 7.78 (dd, 1H, dd, $J = 8.4$, 7.7 Hz, 2-H), 7.71 (m, 2H, AA′ part of AA′XX′), 7.53 (m, 2H, XX′ part of AA′XX′), 7.50 (dd, 1H, $J = 8.4$, 1.1 Hz, 3-H), 5.46 (m, 1H, 1′-H), 5.12 (m, 1H, 7-H), 4.41–4.33 (m, 2H, 5′-H, 3′-H), 3.98 (s, 3H, 4-OCH_3), 3.74 (m, 1H, 4′-H), 3.05 (d, 1H, $J = 18.7$, 10-H_{eq}), 2.97 (d, 1H, $J = 18.7$ Hz, 10-H_{ax}), 2.37 (s, 3H, 14-CH_3), 2.36 (m, 1H, 4′-OH), 2.22–2.08 (m, 3H, 8-H_{eq}, 8H_{ax}, 2′-H_{eq}), 1.82 (m, 1H, 2′-H_{ax}), 1.30 (d, 3H, $J = 6.5$ Hz, 5′-CH_3), 0.32 (s with Sn satellites, 9H, $Sn(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 212.4, 187.3, 187.0, 167.0, 161.3, 156.7, 156.2, 147.8, 136.2, 136.0, 135.9, 134.7, 134.3, 134.2, 126.3, 120.1, 118.6, 111.8, 111.6, 100.9, 70.2, 69.9, 67.3, 56.9, 46.0, 45.9, 35.4, 33.8, 30.3, 25.1, 17.0, 9.3. HRMS: *m/z* calcd for $[C_{37}H_{41}NO_{11}Sn + Na^+]$: 818.1608. Found: *m/z* 818.1587.

4.6. *N*-Succinimidyl-5-bromo-nicotinic acid (21)

Compound **21** was synthesized via the same procedure used for **19**,[16] except that 5-bromo-nicotinic acid was used as the starting material. The crude product was purified by flash chromatography using CH_3CN–CH_2Cl_2 (70:40) as a mobile phase to give **21** as a white solid. $R_f = 0.45$ CH_3CN–CH_2Cl_2; 50:20, 1.06 g, 69%). ^1H NMR spectral data were in accord with those published previously.[16] ^{13}C NMR ($CDCl_3$): δ 168.8, 160.0, 156.6, 149.5, 140.3, 123.2 121.1, 25.9.

4.7. *N*-Succinimidyl-5-(trimethylstannyl)nicotinic acid (22)

This compound was synthesized via the same procedure that was used for the synthesis of **18**,[16] except that *N*-succinimidyl-5-bromo-nicotinic acid **21** (0.59 g, 1.97 mmol) was used as the starting material. The solution was stirred at 85 °C under an argon atmosphere for 2 h until the catalyst turned black. The solvent was evaporated under reduced pressure to dryness at rt. The crude mixture was applied to a flash chromatography column and eluted with CH_3CN–CH_2Cl_2 gradient (100% CH_2Cl_2 → 50% CH_3CN–20% CH_2Cl_2) to give **22** (0.15 g, 20% yield) as a white crystal ($R_f = 0.4$ CH_3CN–CH_2Cl_2; 50:20); ^1H NMR spectral data were in accord with those published previously.[16] ^{13}C NMR ($CDCl_3$): δ 168.8, 160.0, 156.6, 149.5, 140.3, 123.2 121.1, 25.9, −9.3.

4.8. 3′-*N*-5-Trimethylstannyl-nicotinyldaunorubicin (8)

This compound was synthesized using the same procedure used for the synthesis of **6**, except that *N*-succinimidyl-5-(trimethylstannyl)nicotinic acid **22** (19.7 mg,

0.035 mmol) was used as the starting material. The crude product was purified by flash chromatography using CH_3CN–CH_2Cl_2 (50:50) and then CH_3CN–Et_3N (99:1) as mobile phase to give **8** (20.1 mg, 72%) as a red solid, R_f = 0.3 CH_2Cl_2–CH_3CN; 80:20; ^{1}H NMR ($CDCl_3$): δ 14.00, 13.29 (s, 2H, 6,11-OH), 8.80 (m, 1H), 8.69 (m, 1H), 8.11 (m, 1H), 8.06 (dd, 1H, J = 7.7, 1.1 Hz, 1-H), 7.78 (dd, 1H, J = 8.5, 7.7 Hz, 2-H), 7.37 (dd, 1H, J = 8.5, 1.1 Hz, 3-H), 6.47 (d, 1H), 5.55 (m, 1H, 1′-H), 5.30 (m, 1H, 7-H), 4.49 (s, 1H, 9-OH), 4.40 (m, 1H, 3′-H), 4.33 (m, 1H, 5′-H), 4.07 (s, 3H, 4-OCH_3), 3.77 (m, 1H, 4′-H), 3.28 (dd, 1H, J = 18.7, 2.0 Hz, 10-H_{eq}), 2.98 (d, 1H, J = 18.7 Hz, 10-H_{ax}), 2.43 (s, 3H, 14-CH_3), 2.36 (m, 1H, 4′-OH), 2.18–2.03 (m, 2H, 8-H_{eq}, 8H_{ax}), 1.96 (m, 1H, 2′-H_{eq}), 1.88 (m, 1H, 2′-H_{ax}), 1.33 (d, 3H, J = 6.6 Hz, 5′-CH_3), 0.34 (s with Sn satellites, 9H, $Sn(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 212.2, 187.4, 187.0, 165.7, 161.3, 158.1, 156.6, 156.1, 147.4, 142.7 137.7, 135.9, 135.8, 134.7, 134.2, 130.1, 121.3, 120.1, 118.7, 111.8, 111.6, 100.9, 70.4, 69.8, 67.3, 56.9, 46.2, 35.4, 33.8, 30.4, 25.1, 17.0, −9.2. HRMS: *m*/*z* calcd for [$C_{36}H_{40}N_2O_{11}Sn + Na^+$]: 819.1552. Found: *m*/*z* 819.1532.

4.9. 3′-*N*-5-Iodo-nicotinyldaunorubicin (9)

In a round-bottomed flask, compound **8** (14.0 mg, 0.018 mmol) was dissolved in 1 mL of 1% AcOH/MeOH. Then 105 μL of NaI (5.27 mg, 0.35 mmol) in MeOH was added, followed by 0.56 mL chloramine-T (0.04 g/mL MeOH). The reaction mixture was stirred vigorously and the color changed from red to slightly yellow after 13 min. After this, the reaction was quenched by the addition of 0.84 mL of sodium metabisulfite (0.04 mg/mL in H_2O), which gave the original color, red, again. The solvent was evaporated under reduced pressure to dryness at rt. The crude product was purified by flash chromatography using 100% EtOAc as a mobile phase to give **9** (10.3 mg, 77%) as slightly red solid; ^{1}H NMR ($CDCl_3$): δ 14.00, 13.30 (s, 2H, 6,11-OH), 8.91 (m, 1H), 8.89 (m, 1H), 8.34 (m, 1H), 8.06 (dd, 1H, J = 7.7, 1.1 Hz, 1-H), 7.78 (dd, 1H, J = 8.5, 7.7 Hz, 2-H), 7.37 (dd, 1H, J = 8.5, 1.1 Hz, 3-H), 6.43 (d, 1H), 5.54 (m, 1H, 1′-H), 5.32 (m, 1H, 7-H), 4.43 (s, 1H, 9-OH), 4.39–4.30 (m, 2H, 5′-H, 3′-H), 4.07 (s, 3H, 4-OCH_3), 3.74 (m, 1H, 4′-H), 3.29 (dd, 1H, J = 19.0, 2.0 Hz, 10-H_{eq}), 2.98 (d, 1H, J = 19.0 Hz, 10-H_{ax}), 2.43 (s, 3H, 14-CH_3), 2.35 (m, 1H, 4′-OH), 2.14–2.04 (m, 2H, 8-H_{eq}, 8-H_{ax}), 1.96 (m, 1H, 2′-H_{eq}), 1.86 (m, 1H, 2′-H_{ax}), 1.33 (d, 3H, J = 6.6 Hz, 5′-CH_3); ^{13}C NMR ($CDCl_3$): δ 212.1, 187.4, 187.0, 163.6, 161.3, 158.5, 156.6, 156.1, 146.5, 143.4, 135.9, 135.8, 134.7, 134.1, 121.3, 120.1, 118.7, 111.8, 111.6, 100.8, 70.4, 69.7, 67.2, 56.9, 46.3, 35.4, 33.7, 30.3, 25.0, 16.9. HRMS: *m*/*z* calcd for [$C_{33}H_{31}IN_2O_{11} + Na^+$]: 781.0870. Found: *m*/*z* 781.0869.

4.10. 3′-*N*-(4-Hydroxybenzyl)daunorubicin (12) and 3′-*N*-(4-hydroxybenzyl)-13-(*R*/*S*)-dihydrodaunorubicin (15)

A mixture of hydrochloride salt of daunorubicin **1a** (25 mg, 0.044 mmol), and 4-hydroxybenzaldehyde (16 mg, 0.13 mmol) was dissolved in acetonitrile–water (2:1, 6 mL). To the stirred solution was added sodium cyanoborohydride (2.4 mg, 0.04 mmol, 1 M solution in THF). The reaction mixture was stirred under a nitrogen atmosphere at rt in the dark for 24 h, and the consumption of the daunorubicin was followed by TLC. Additional 4-hydroxyaldehyde (22 mg) and sodium cyanoborohydride (4 mg) were added successively. The reaction was stirred for another 2 days. The solvent was evaporated and the crude mixture was purified by flash chromatography. Two fractions were isolated and analyzed by NMR and LC/MS. The first fraction (6 mg, 22%) was found to be compound **12**. The second product (21 mg, 75%) was analyzed and found to be compound **15** (as a mixture of two diastereomers).

4.10.1. Data for 3′-*N*-(4-hydroxybenzyl)daunorubicin (12). ^{1}H NMR (CD_3OD): δ 7.93 (d, 1H, H-3), 7.82 (t, 1H, H-2), 7.56 (d, 1H, H-1), 7.20 (d, 2H, Ar), 6.71 (d, 2H, Ar), 5.46 (br s, 1H, H-1′), 5.09 (br s, 1H, H-7), 4.27 (q, 1H, H-5′), 3.90 (s, 3H, OCH_3), 3.63 (br s, 1H, H-4′), 3.67–3.48 (m, 3H, H-3′, H-10$_{eq}$, H-10$_{ax}$), 2.99 (m, 2H, CH_2), 2.35 (s, 3H, $COCH_3$), 2.33–2.10 (m, 2H, H-8), 2.05–1.90 (m, 2H, H-2′), 1.29 (d, 3H, 5′-CH_3); ^{13}C NMR (CD_3OD): δ 213.4, 203.7, 164.3, 160.9, 137.2, 132.3, 121.9, 120.6, 120.5, 116.7, 113.1, 101.3, 97.3, 74.6, 71.8, 68.4, 66.2, 57.1, 54.6, 37.0, 33.2, 29.1, 24.5, 17.1. HRMS: *m*/*z* calcd for [$C_{34}H_{35}NO_{11} + H^+$]: 634.2288. Found: *m*/*z* 634.2270.

4.10.2. Data for 3′-*N*-(4-hydroxybenzyl)-13-(*R*/*S*)-dihydrodaunorubicin (15). ^{1}H NMR (CD_3OD): δ 7.77 (m, 2H, H-3, H-2), 7.48 (d, 1H, H-1), 7.01 (2H, Ph), 6.82 (2H, Ph), 6.54 (2H, Ph), 6.37 (2H, Ph), 5.45 (br s, 1H, H-1′), 5.00 (br s, 1H, H-7), 4.25 (q, 1H, H-5′), 3.98 (s, 3H, OCH_3), 3.79 (m, 2H, H-14, H-4′), 3.68–3.47 (m, 3H, CH_2, H-3′), 2.93 (m, 1H, H-10), 2.53 (m, 2H, H-10, H-8), 2.17 (m, 1H, H-8), 1.96 (m, 2H, H-2′), 1.92–1.33 (m, 6H, $COCH_3$, CH_3); ^{13}C NMR (CD_3OD): δ 213.0, 188, 162.4, 159.3, 157.0, 137.0, 136.3, 136.2, 132.2, 132.1, 123.5, 120.9, 120.2, 116.6, 116.4, 112.4, 112.0, 101.1, 74.2, 73.9, 72.7, 71.8, 68.0, 65.9, 57.0, 54.6, 35.9, 33.3, 32.2, 29.2, 28.9, 17.2, 16.8. HRMS: *m*/*z* calcd for [$C_{34}H_{37}NO_{11} + H^+$]: 636.2445. Found: *m*/*z* 636.2418.

4.11. 3′-*N*-(4-Hydroxy-3-iodobenzyl)daunorubicin (11) and 3′-*N*-(4-hydroxy-3-iodobenzyl)-13-(*R*/*S*)-dihydrodaunorubicin (14)

To a solution of daunorubicin hydrochloride (30 mg, 0.053 mmol) and 4-hydroxy-3-iodobenzaldehyde

(39.6 mg, 0.16 mmol) in a 2:1 ratio mixture of acetonitrile and water (8 mL) was added 1 M THF solution of sodium cyanoborohydride (3.4 mg, 0.053 mmol). The reaction mixture was stirred at rt in the dark under a nitrogen atmosphere for 24 h and the progress of the reaction was monitored by TLC. Additional aldehyde (20 mg) and sodium cyanoborohydride (7 mg) were added successively and the reaction mixture was stirred for another 2 days. The solvent was evaporated and the crude mixture was purified by flash chromatography. The reaction produced **11** (15 mg, 37%) and **14** (20 mg, 50%, mixture of two diastereomers).

4.11.1. Data for 3′-*N*-(4-hydroxy-3-iodobenzyl)daunorubicin (11). ^{1}H NMR (CD_3OD): δ 7.88 (d, 1H, H-3), 7.81 (t, 1H, H-2), 7.72 (s, 1H, Ph), 7.53 (d, 1H, H-1), 7.20 (d, 1H, Ph), 6.76 (d, 1H, Ph), 5.46 (br s, 1H, H-1′), 5.05 (br s, 1H, H-7), 4.28 (m, 1H, H-5′), 4.01 (s, 3H, OCH_3), 3.86 (br s, 1H, H-4′), 3.50 (m, 1H, H-3′), 3.22 (m, 1H, H-10), 3.00 (m, 2H, CH_2), 2.46 (m, 1H, H-10), 2.36 (s, 3H, $COCH_3$), 2.01 (m, 2H, H-8), 1.79 (m, 1H, H-2′), 1.62 (m, 1H, H-2′), 1.30 (d, 3H, 5′-CH_3); ^{13}C NMR (CD_3OD): δ 213.4, 203.7, 162.5, 159.2, 154.9, 142.1, 132.4, 120.5, 120.3, 115.8, 115.7, 101.2, 84.9, 77.5, 73.2, 67.9, 66.1, 57.1, 56.0, 37.0, 31.5, 30.6, 28.7, 24.5, 17.0. HRMS: *m*/*z* calcd for [$C_{34}H_{34}INO_{11} + H^+$]: 760.1255. Found: *m*/*z* 760.1286.

4.11.2. Data for 3′-*N*-(4-hydroxy-3-iodobenzyl)-13-(*R*/*S*)-dihydrodaunorubicin (14). ^{1}H NMR (CD_3OD): δ 7.93 (m, 1H, H-3), 7.82 (m, 1H, H-2), 7.64 (s, 1H, Ph), 7.55 (m, 1H, H-1), 7.1 (d, 2 H, J = 7.6 Hz, Ph), 6.96 (d, 2 H, J = 7.6 Hz, Ph), 6.67 (d, 2 H, J = 7.6 Hz, Ph), 6.57 (d, 2 H, J = 7.6 Hz, Ph), 5.50 (s, 1H, H-1′), 5.10 (br s, 1H, H-7), 4.28 (m, 1H, H-5′), 4.02 (s, 3H, OCH_3), 3.83 (br s, 2H, CH_2Ph), 3.71 (m, 1H, H-4′), 3.67 (m, 1H, H-3′), 3.47 (m, 1H, H-13), 3.11 (m, 1H, H-13), 3.08 (d, 1H, J = 18.7 Hz, H-10), 2.99 (d, 1H, J = 18.7 Hz, H-10), 2.71 (d, 1H, J = 18.7 Hz, H-10), 2.61 (d, 1H, J = 18.7 Hz, H-10), 2.54 (m, 1H, H-8), 2.22 (m, 1H, H-8), 2.15–1.74 (m, 3H, H-8, H-2′), 1.35–1.28 (m, 6H, 14-CH_3, 5′-CH_3); ^{13}C NMR (CD_3OD): δ 213.0, 188.0, 187.9, 162.5, 159.2, 159.0, 157.1, 157.0, 142.0, 137.2, 136.3, 136.2, 136.1, 136.0, 132.4, 125.0, 121.5, 121.4, 120.6, 120.4, 115.6, 115.4, 112.5, 112.2, 101.1, 84.9, 74.2, 73.9, 73.2, 72.8, 72.3, 57.1, 54.9, 35.9, 33.8, 32.3, 30.6, 28.9, 28.8, 17.2, 17.1, 16.8. HRMS: *m*/*z* calcd for [$C_{34}H_{36}INO_{11} + H^+$]: 762.1411. Found: *m*/*z* 762.1435.

4.12. General ^{125}I-labeling procedure

The following stock solutions were prepared: daunorubicin/doxorubicin derivatives (1 and 4 mg/mL) in MeOH solution. *N*-Chloro-*p*-toluenesulfonamide sodium salt (4 and 8 mg/mL) was dissolved in MeOH prior to use. In a typical labeling experiment, 3.7 GBq/μL of [^{125}I] iodide was used.

To 40 μL of MeOH solution of daunorubicin derivative, 5.0 μL aqueous ^{125}I-solution was added, followed by 10 μL CAT solution. The reaction mixture was vortexed for 5 min, and quenched by the addition of 10 μL sodium metabisulfite solution; 20 μL of NaI (10 mg/mL solution in H_2O) solution was added to stabilize [^{125}I]-iodide against oxidation during the TLC analysis. Exact concentrations of solutions are given in the figure legends. After preparing the reaction mixture, samples for radio-TLC analysis (1–2 μL) were collected. Blank experiments were run using exactly the same conditions, but neat MeOH solution was used instead of the stock solution of daunorubicin/doxorubicin derivatives. All blank experiments were tested in both TLC systems.

The crude product was purified using C18 solid phase extraction, eluting any unreacted [^{125}I] iodide with water and the reaction product with 100% ethanol.

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