7.20–8.05 (m, 3 H, aromatic). Anal. ($C_{31}H_{35}NO_{11}S$·HCl·0.5H_2O) C, H, N, S.

Adriamycin 14-Thiovalerate (4d). Thiovaleric acid (100 μL) was added to a stirred mixture of **5** (300 mg, 0.47 mmol) and anhydrous K_2CO_3 (75 mg) in methanol (30 mL) at room temperature. The product, worked up as above, was chromatographed on Biosil A (9 g). $CHCl_3$–MeOH (2%) eluted pure **4d**; the hydrochloride salt (225 mg, 72%) was homogeneous on TLC (B, R_f 0.44) and HPLC (t_r = 6.15 min); NMR of free base δ 0.97 (t, J = 6 Hz, 3 H, S $CO(CH_2)_3CH_3$), 1.32 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.05 (s, 3 H, Ar OCH_3), 5.23 (m, 1 H, 7-H), 5.50 (m, 1 H, 1′-H), 7.20–7.95 (m, 3 H, aromatic). Anal. ($C_{32}H_{37}NO_{11}S$·HCl·0.5H_2O) C, H, N, S.

Adriamycin 14-Thiobenzoate (4e). Compound **5** (150 mg, 0.24 mmol) and K_2CO_3 (50 mg) were stirred in methanol (20 mL) at room temperature. Thiobenzoic acid (29 μL, 0.27 mmol) was added and stirring was continued for 10 min. The product, worked up as usual, was chromatographed on Biosil A (5 g). $CHCl_3$–MeOH (2%) eluted pure **4e**; hydrochloride salt (121 mg, 74%) homogeneous on TLC (B, R_f 0.38) and HPLC (t_r = 6.19 min); NMR of free base δ 1.32 (d, J = 6 Hz, 3 H, 5′-CH_3), 4.01 (s, 3 H, Ar OCH_3), 5.18 (m, 1 H, 7-H), 5.45 (m, 1 H, 1′-H), 7.25–8.04 (m, 8 H, aromatic). Anal. ($C_{34}H_{33}NO_{11}S$·HCl·2.5H_2O) C, H, N, S.

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Registry No. **3a**, 101980-69-2; **3b**, 101980-70-5; **3c**, 101980-71-6; **3d**, 101980-72-7; **3e**, 101980-73-8; **4a**, 102045-67-0; **4a**·HCl, 65208-77-7; **4b**, 101980-74-9; **4b**·HCl, 102045-68-1; **4c**, 101980-75-0; **4c**·HCl, 102045-69-2; **4d**, 101980-76-1; **4d**·HCl, 102129-51-1; **4e**, 102045-70-5; **4e**·HCl, 65208-78-8; **5**, 65026-79-1; **6**, 77270-18-9; thioacetic acid, 507-09-5; thiopropionic acid, 1892-31-5; thiobutyric acid, 3931-64-4; thiovaleric acid, 53966-59-9; thiobenzoic acid, 98-91-9.

Adriamycin Analogues. Preparation and Biological Evaluation of Some *N*-(Trifluoroacetyl)-14-*O*-[(*N*-acetylamino)acyl]adriamycin Derivatives[1]

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In connection with structure–activity studies related to the novel DNA-nonbinding adriamycin analogues *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32) and *N*-(trifluoroacetyl)adriamycin 14-*O*-hemiadipate (AD 143), we have now prepared a series of *N*-(trifluoroacetyl)adriamycin derivatives with *N*-acylamino acid esters at the 14-carbinol position. Target compounds were made by reaction of *N*-(trifluoroacetyl)-14-iododaunorubicin with the sodium salts of *N*-acylamino acids generally in dimethylformamide–ethylene glycol solvent. Products were evaluated for in vitro growth-inhibitory activity and, to a limited extent, in vivo antitumor activity in the murine P388 leukemia system. ID_{50} values for the target compounds vs. cultured CCRF-CEM cells were generally in the same range as those for the above-mentioned DNA nonbinding adriamycin analogues. Of the four compounds tested for in vivo activity, although none was as effective as *N*-(trifluoroacetyl)adriamycin 14-valerate, all showed significant activity in the P388 assay system, with three of the compounds, at the doses used, being essentially equiactive with an optimal dose of adriamycin. Studies on the rate of esterase-mediated deacylation of the products, in a defined system containing unfractionated mouse serum as the source of enzyme, showed no relationship between the in vitro and in vivo activities of these compounds and the relative ease at which the side-chain ester substituents were hydrolyzed.

A major program in these laboratories directed toward anthracycline chemistry and pharmacology has resulted, among other accomplishments, in the development of certain novel DNA-nonbinding *N*-(trifluoroacetyl)adriamycin 14-*O*-alkanoate esters with therapeutic properties superior in animals and humans to those of the widely used antitumor antibiotics daunorubicin (**1**) and adriamycin (doxorubicin, **2**). Compounds of this class of greatest clinical interest include *N*-(trifluoroacetyl)adriamycin 14-valerate (**3**) and *N*-(trifluoroacetyl)adriamycin 14-*O*-hemiadipate (**4**), as summarized in a recent paper appearing in this Journal.[2]

As part of our effort to explore the unusual properties of these agents, and perhaps identify additional analogues of potential therapeutic value, we have been examining diverse structural variants of **3** and **4**, with the view toward developing structure–activity relationships for this system.[3-9] In this regard, it is known that the initial metabolic processing of **3** and **4** in man, laboratory animals, and cell culture systems involves enzyme-mediated loss of the 14-*O*-acyl function, with consequent formation of *N*-(trifluoroacetyl)adriamycin (**5**), an event brought about by the action of ubiquitously occurring nonspecific serum and tissue esterases.[10-15] The present report concerns the

Scheme I

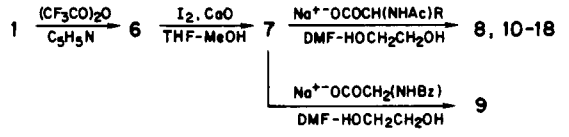

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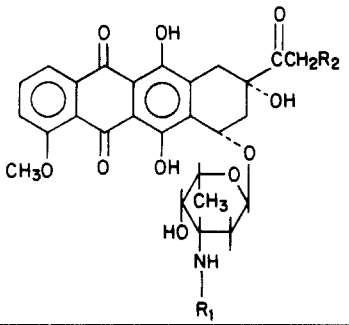
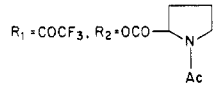
Table I. Structures



no.	R groups
1	$R_1 = R_2 = H$ (daunorubicin)
2	$R_1 = H$, $R_2 = OH$ (adriamycin)
3	$R_1 = COCF_3$, $R_2 = OCO(CH_2)_3CH_3$ (AD 32)
4	$R_1 = COCF_3$, $R_2 = OCO(CH_2)_4COOH$ (AD 143)
5	$R_1 = COCF_3$, $R_2 = OH$
6	$R_1 = COCF_3$, $R_2 = H$
7	$R_1 = COCF_3$, $R_2 = I$
8	$R_1 = COCF_3$, $R_2 = OCOCH_2NHAc$
9	$R_1 = COCF_3$, $R_2 = OCOCH_2NHCOC_6H_5$
10	$R_1 = COCF_3$, $R_2 = OCOCH(NHAc)CH_3$
11	$R_1 = COCF_3$, $R_2 = OCOCH(NHAc)CH_2C_6H_5$
12	$R_1 = COCF_3$, $R_2 = OCOCH(NHAc)CH_2CH_2SCH_3$
13	$R_1 = COCF_3$, $R_2 = OCOCH(NHAc)CH(CH_3)CH_3$
14	$R_1 = COCF_3$, $R_2 = OCOCH(NHAc)CH_2CH(CH_3)CH_3$
15	
16	$R_1 = COCF_3$, $R_2 = OCOCH_2CH(NHAc)COOH$
17	$R_1 = COCF_3$, $R_2 = OCOCH_2CH_2CH(NHAc)COOH$
18	$R_1 = COCF_3$, $R_2 = OCOCH_2CH_2CH(NHAc)CONH_2$

synthesis and biological evaluation of some 14-*O*-(*N*-acylamino) acid ester analogoues of **3** and **4**, which have been designed to investigate the effect of a bulky substituent adjacent to the active site of enzymatic deacylation. Structures of the products (**8–18**) involved in this study are shown in Table I. Of the 11 compounds prepared in connection with this work, 10 are *N*-acetylamino acid derivatives, seven of which have the acetylamino function α to the ester group. The *N*-acetylamino derivatives of aspartic acid, glutamic acid, and glutamine (**16**, **17**, and **18**, respectively) are attached to the anthracycline through the carboxyl functions on the other side of the molecule from the α-acetylamino group. The *N*-benzoylglycine derivative **9** was made for comparison with the corresponding *N*-acetyl product **8**, to examine the effect of a different large substituent at the α-amino position.

The preparation of the target compounds was straightforward and followed the general approach used here previously to obtain **3** and related products. The synthetic route is outlined in Scheme I. Daunorubicin (**1**) was converted in quantitative yield into its trifluoroacetamide **6** by means of trifluoroacetic anhydride in pyridine; this is an improved procedure to that of the patent literature, in which chloroform–ether is suggested as solvent.[16] Compound **6**, on treatment with iodine in the presence of dry calcium oxide, afforded the 14-iodo *N*-trifluoroacetamide **7**, as previously described.[16] Reaction of **7**, generally in dimethylformamide–ethylene glycol, with the sodium salts of *N*-acylamino acids (prepared by carefully titrating the amino acid derivative with dilute sodium hydroxide and then lyophilizing the mixture) gave the desired compounds. All products were purified to homogeneity, as determined by thin-layer and high-performance liquid chromatography, and characterized by microchemical analyses and spectral properties. The structures of all compounds are unambiguous, except for **16** and **17**, which are assumed to be as shown, based on the consideration that the non-amino acid carboxyl of the dibasic acids is the more acidic of the two acid functions and would be the preferred site for salt formation when carefully titrated with 1 equiv of base.

Table II. In Vitro Biological Evaluation of Some 14-*O*-(*N*-Acylamino) Acid Esters of *N*-(Trifluoroacetyl)adriamycin (**5**)

no.	$T_{1/2}$,[a] min	ID_{50},[b] μM	no.	$T_{1/2}$,[a] min	ID_{50},[b] μM
8	10	0.14	**14**	65	0.15
9	3.5	0.12	**15**	*d*	0.30
10	18	0.15	**16**	18	1.00
11	14	0.09	**17**	16	0.16
12	38	0.83	**18**	24	0.12
13	*c*	0.14			

[a] Time of enzyme-mediated conversion of 50% of test compound to **5**; pH 7.0 carbonate buffer supplemented with 1% unfractionated mouse serum, 37 °C incubation. [b] Versus CCRF-CEM cells in culture; 48-h continuous drug exposure. [c] 8% hydrolysis in 60 min. [d] 4% hydrolysis in 60 min.

In vitro biological evaluation of the target compounds involved growth-inhibition assays with cultured human-derived leukemic lymphocytes and studies with unfractionated mouse serum on the relative rate of esterase-mediated side-chain deacylation; the data are provided in Table II.

To determine the rate at which deacylation occurred, use was made of sensitive liquid chromatographic assay procedures, which monitored simultaneously the disappearance of the test compound and the appearance of the hydrolysis product **5** under defined experimental conditions. Serum was collected and pooled from 10 B6D2F1 male mice, sacrificed by cervical dislocation, and was maintained frozen at −70 °C, except as needed. Test compounds were added at equimolar concentrations to a specified volume of carbonate buffer, pH 7.0, supplemented with 1% of the pooled mouse serum. The mixtures were incubated at 37 °C and sampled at 5-min intervals for determination of the amount of test compound remaining and the amount of **5** formed. $T_{1/2}$ values (time of 50% conversion) were extrapolated from curves constructed from the data points. Under these conditions, **3** has been shown to have a $T_{1/2}$ of 10 min.[10] For the (*N*-acetylamino)acyl compounds tested, the relative rate of hydrolysis was influenced by the structure of the particular ester attachment, although precise structure–activity relationships do not appear capable of being generalized. Compound **8** has a $T_{1/2}$ identical with that of **3**. The other *N*-acetyl derivatives show values that varied from somewhat to very much longer than **3** in terms of their ability to be deacylated under these conditions. The compound least susceptible to hydrolysis was the cyclic proline de-

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rivative **15**, which underwent deacylation only to the extent of 4% over 60 min. Two other derivatives showing markedly slow deacylation were the branched-chain *N*-acetylvaline and *N*-acetylleucine esters of **5**, compounds **13** and **14**, respectively. When the branched methyl group was adjacent to the acetamide, as in **13**, hydrolysis proceeded to only 8% by 60 min (13% at the end of 120 min), whereas when the branched methyl function was located somewhat further down the chain, as in **14**, deacylation occurred more rapidly (50% in 65 min) but still relatively slowly compared with the various staight-chain esters. Interestingly, the *N*-benzoylglycine ester **9** underwent deacylation 3 times more rapidly than did the corresponding *N*-acetylglycine derivative **8**. Although closely analogous in structure with respect to the ester linkage, the *N*-acetylglutaminyl derivative **18** was hydrolyzed 50% more slowly than the corresponding *N*-acetylglutamate **17**.

Growth-inhibition assays were performed with CCRF-CEM cells in suspension culture in Eagle's minimal essential media supplemented with 10% fetal bovine serum. The cell line is derived from the peripheral blood buffy coat of a child who presented clinically with lymphocytic leukemia some years ago. The development of this immortalized cell line and assay procedures for its use have been described previously.[17,18] The ID_{50} value represents the concentration of drug needed to bring about a 50% reduction in the growth of treated cultures relative to untreated controls. In this system the ID_{50} for **2** generally runs in the range of 0.03–0.06 μM. Compound **3** has reproducibly exhibited an ID_{50} value of 0.23–0.24 μM, relative to **2** at 0.06 μM. As seen in Table II, the ID_{50} values for most of the target compounds involved in this study were in the same range as that for **3**. Only the *N*-acetylmethionyl and *N*-acetylaspartyl derivatives (**12** and **16**, respectively) were clearly less active; interestingly, these compounds are the only ones of the series to have additional functionality two carbons removed from the α-acetamido group. For the compounds tested, no relationship appears to exist for growth-inhibitory activity relative to the ease of ester hydrolysis, as brought about by esterases in unfractionated mouse serum: the slowest derivatives to hydrolyze (**13–15**) were as inhibitory of the growth of CEM cultures as were the most rapidly hydrolyzed. This suggests that the hydrolysis of **3** and related compounds by mouse serum esterases may be less important to the pharmacologic effects exhibited by these agents than the *intracellular* conversion of **3** into **5**, which also occurs, especially in liver and kidney.

Four of the title compounds were additionally tested for in vivo antileukemia activity in the murine P388 tumor model system. These assays were performed essentially according to the standard protocols established by the National Cancer Institute, with 10^6 tumor cell inoculum injected into B6D2F1 male mice intraperitoneally on day 0 and treatment administered intraperitoneally once daily commencing on day 1.[19] Treatments were administered for 4 days, rather than the 9 days suggested in the published protocols, as we have found this modified schedule with **2** and related compounds to be more sparing of toxicity, with resultant higher percent increase in life span (ILS) data for responding animals. At the highest dose tested (50 mg/kg per day in each instance), compound **8** showed good antitumor activity, with a +105% ILS. The other three compounds were essentially equiactive with **2**, **10** affording a +163% ILS; **11**, +173% ILS; and **12**, +136% ILS. Compound **2**, run as a positive control in these assays, at an optimal dose of 2.0 mg/kg per day for 4 days produced a +145% ILS. There were no long-term survivors (>40 days) in any of the treatment groups receiving **2** or any of the test compounds. However, compound **3**, also used as a positive control in these assays, at doses of both 40 and 50 mg/kg per day for 4 days produced, in each instance, 100% curves, with 7/7 animals at each dose level alive and tumor-free on day 75. Because none of the test compounds, although active, afforded activity at the highest dose tested anywhere near that exhibited by lesser quantities of **3**, the remaining (*N*-acetylamino)acyl derivatives were not evaluated in this test system. Interest still exists, however, in assaying these derivatives in various murine solid tumor model systems, wherein possible therapeutic advantages may be expressed as a result of differential behavior of these agents to tissue esterases and amidases.

Experimental Section

Except for *N*-acetylproline, which was a racemic mixture, the *N*-acylamino acids used in this work were all of the natural L configuration; all of the starting amino acid derivatives were obtainable commercially. Homogeneity of products was checked by both TLC [silica gel G plates (Analtech), $CHCl_3$–MeOH, 10:1, as irrigant] and HPLC [2 ft × $^1/_8$ in. o.d. column packed with phenyl/Corasil (Waters Associates) run in reverse phase; acetonitrile–pH 4.0 ammonium formate buffer, gradient elution, 32–65% acetonitrile over 6 min at a flow rate of 3.5 mL/min; fluorescence detection, excitation at 482 nm]. Infrared spectra were recorded as KCl pellets on Perkin-Elmer Model 137B and 1320 spectrophotometers and show characteristic bands for the anthracycline nucleus, including 3500 (OH), 1724 ($COCF_3$, $COCH_2$, $NCOCH_3$), 1620, 1580 (quinone) cm^{-1}. UV–vis spectra were recorded in MeOH on a Perkin-Elmer Lambda 3B spectrophotometer, interfaced with their Model 3600 Data Station. Proton NMR spectra were recorded on a Varian EM360-L spectrometer in $CDCl_3$, unless otherwise noted, with tetramethylsilane as internal standard. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN; found values are within ±0.4% of theory, unless otherwise indicated.

***N*-(Trifluoroacetyl)daunorubicin (6).** Daunorubicin (1) hydrochloride (1.6 g, 2.6 mmol) was stirred in dry pyridine (16 mL) at −20 °C (bath temperature; CCl_4/dry ice bath) for 15 min. Trifluoroacetic anhydride (2.8 mL) in anhydrous ether (20 mL) was added dropwise over a 5-min period. After 10 min, water (25 mL) was added and stirring was continued for 15 min more. The reaction mixture was extracted with ethyl acetate (2 × 100 mL), and the extracts were washed with water (5 × 25 mL). After drying (Na_2SO_4), the ethyl acetate solution was filtered and evaporated to dryness, and the residue was heated at reflux with MeOH (100 mL) for 10 min. MeOH was distilled off on the rotary evaporator, and the residue was precipitated from $CHCl_3$–petroleum ether to afford **6** (1.75 g, 99%), identical in all respects with authentic material.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylglycyl)adriamycin (8).** *N*-Acetylglycine sodium salt (400 mg, 3.45 mmol) was dissoved in ethylene glycol (4 mL), and to this solution was added *N*-(trifluoroacetyl)-14-iododaunorubicin (**7**;[16] 400 mg, 0.53 mmol) dissolved in DMF (8 mL). The mixture was heated at 50 °C for 1.5 h and then was taken in $CHCl_3$ (50 mL) and washed with water (5 × 50 mL). The $CHCl_3$ solution was dried (Na_2SO_4), filtered, and evaporated to dryness, and the residue precipitated from $CHCl_3$-petroleum ether to obtain pure **8** (310 mg, 78%); homogeneous on TLC (R_f 0.47) and HPLC (t_r = 4.95 min); UV–vis λ_{max} (ϵ) 232 (41 435), 250 (26 695), 285 (10 540), 476 (11 945), 494 (11 945), and 530 (7090) nm; NMR ($CDCl_3$–CD_3OD) δ 1.32 (d, J = 6.5 Hz, 5′-CH_3), 2.06 (s, 3 H, $NHCOCH_3$), 4.06 (s, 3 H, OCH_3), 5.33 (br s, 3 H, 7-H and 14-H_2), 5.55 (m, 1 H, 1′-H), 7.28–8.00 (m, 3 H, aromatic). Anal. ($C_{33}H_{33}F_3N_2O_{14}\cdot H_2O$) C, H, N; F: calcd, 7.53; found, 6.51.

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Table III. Reaction Conditions and Chromatographic Properties for Products 9–18

no.	reaction conditions: solvent[a]	time, min	temp, °C	yield, %	TLC,[b] R_f	HPLC,[b] t_r, min
9	A + B	60	45	38	0.36	5.32
10	B	5	37	95	0.47	5.81
11	A + B	60	36	55	0.46	5.48
12	B	45	25	74	0.40	5.20
13	A + B	90	37	56	0.47	6.45
14	A	90	37	90	0.34	6.96
15	A + B	90	40	81	0.40	6.30
16	A	15	40	31	0.45	3.95
17	A + B	35	40	40	0.46	4.01
18	A + B	50	45	56	0.22	4.18

[a] A = ethylene glycol; B = dimethylformamide. [b] See Experimental Section for separation conditions.

Experimental details for compound 8 may be considered as a general procedure for 9–18; differences in reaction conditions, yield, and chromatographic properties are given in Table III. Spectral and analytical data for compounds 9–18 are provided below.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-benzoylglycyl)adriamycin (9)**: UV–vis λ_{max} (ϵ) 232 (50 015), 248 (30 105), 287 (10 840), 480 (11 950), 494 (12 005), and 529 (7,270) nm; NMR δ 1.35 (d, J = 6.0 Hz, 3 H, 5′-CH_3), 3.98 (s, 3 H, OCH_3), 7.2–8.0 (m, 8 H, aromatic). Anal. ($C_{38}H_{35}F_3N_2O_{14}\cdot 0.5H_2O$) C, H, N; F: calcd, 7.04; found, 6.55.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylalanyl)adriamycin (10)**: UV–vis λ_{max} (ϵ) 232 (42 650), 250 (29 570), 287 (11 840), 480 (10 890), 497 (11 250), and 532 (8900) nm; NMR δ 1.30 (d, J = 6.5 Hz, 3 H, 5′-CH_3), 1.55 (d, J = 6.5 Hz, 3 H, NCHCH_3), 2.02 (s, 3 H, $NCOCH_3$), 4.00 (s, 3 H, OCH_3), 5.23 (br s, 3 H, 7-H and 14-H_2), 5.47 (m, 1 H, 1′-H), 6.23 (d, 1 H, J = 8 Hz, N*H*$COCH_3$), 7.20–7.90 (m, 3 H, aromatic). Anal. ($C_{34}H_{35}F_3N_2O_{14}$) C, H, N; F: calcd, 7.57; found, 6.73.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylphenylalanyl)adriamycin (11)**: UV–vis λ_{max} (ϵ) 232 (43 770), 250 (30 410), 287 (11 935), 480 (10 570), 497 (11 445), and 532 (9440) nm; NMR δ 1.30 (d, J = 6.5 Hz, 3 H, 5′-CH_3), 2.00 (s, 3 H, $NCOCH_3$), 4.03 (s, 3 H, OCH_3), 5.23 (m, 3 H, 7-H and 14-H_2), 5.50 (m, 1 H, 1′-H), 6.20 (d, J = 8 Hz, 1 H, N*H*$COCH_3$), 7.30–7.95 (m, 3 H, aromatic). Anal. ($C_{40}H_{39}F_3N_2O_{14}\cdot H_2O$) C, H, N; F: calcd, 6.73; found, 6.27.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylmethionyl)adriamycin (12)**: UV–vis λ_{max} (ϵ) 232 (45 895), 250 (31 940), 287 (11 645), 480 (12 760), 496 (13 545), and 531 (10 275) nm; NMR δ 1.33 (d, J = 6.5 Hz, 3 H, 5′-CH_3), 2.07 (s, 3 H, SCH_3), 2.12 (s, 3 H, $NCOCH_3$), 4.04 (s, 3 H, OCH_3), 5.33 (br s, 3 H, 7-H and 14-H_2), 5.57 (m, 1 H, 1′-H), 6.40 (d, J = 6 Hz, 1 H, N*H*$COCH_3$), 6.90 (d, J = 9 Hz, 1 H, $NHCOCF_3$), 7.23–8.02 (m, 3 H, aromatic). Anal. ($C_{36}H_{39}F_3N_2O_{14}S\cdot H_2O$) C, H, F, N, S.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylvalyl)adriamycin (13)**: UV–vis λ_{max} (ϵ) 232 (42 640), 250 (29 260), 287 (11 000), 480 (12 310), 495 (12 505), and 530 (7965) nm; NMR δ 1.05 [d, J = 7 Hz, 6 H, $CH(CH_3)_2$], 1.37 (d, J = 6 Hz, 3 H, 5′-CH_3), 2.12 (s, 3 H, $NCOCH_3$), 4.04 (s, 3 H, OCH_3), 5.33 (br s, 3 H, 7-H and 14-H_2), 5.57 (m, 1 H, 1′-H), 6.28 (d, J = 9 Hz, 1 H, N*H*$COCH_3$), 7.17 (d, J = 9 Hz, 1 H, $NHCOCF_3$), 7.33–8.0 (m, 3 H, aromatic). Anal. ($C_{36}H_{39}F_3N_2O_{14}\cdot 0.5H_2O$) C, H, F, N.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylleucyl)adriamycin (14)**: UV–vis λ_{max} (ϵ) 232 (43 995), 250 (29 060), 287 (10 425), 480 (12 955), 494 (12 900), and 529 (7480) nm; NMR δ 1.00 [d, J = 5 Hz, 6 H, $CH(CH_3)_2$], 1.35 (d, J = 6 Hz, 3 H, 5′-CH_3) 2.07 (s, 3 H, $NCOCH_3$), 4.03 (s, 3 H, OCH_3), 5.27 (br s, 3 H, 7-H and 14-H_2), 5.50 (m, 1 H, 1′-H), 6.12 (d, J = 9 Hz, 1 H, $NHCOCH_3$), 6.97 (d, J = 9 Hz, 1 H, $NHCOCF_3$), 7.26–7.97 (m, 3 H, aromatic). Anal. ($C_{37}H_{41}F_3N_2O_{14}\cdot 0.5H_2O$) C, H, F; N: calcd, 3.48; found, 2.99.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylprolyl)adriamycin (15)**: UV–vis λ_{max} (ϵ) 232 (39 535), 250 (26 865), 285 (10 605), 480 (10 440), 494 (10 475), and 528 (6085) nm; NMR δ 1.35 (d, J = 6 Hz, 3 H, 5′-CH_3), 2.10 (s, 3 H, $NCOCH_3$), 4.05 (s, 3 H, OCH_3), 5.24 (br s, 3 H, 7-H and 14-H_2), 5.50 (br s, 1 H, 1′-H), 7.07 (d, J = 9 Hz, 1 H, $NHCOCF_3$), 7.33–8.08 (m, 3 H, aromatic). Anal. ($C_{36}H_{37}F_3N_2O_{14}\cdot 0.5H_2O$) C, H, F, N.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetyl-4-aspartyl)adriamycin (16)**: UV–vis λ_{max} (ϵ) 232 (39 340), 250 (26 625), 290 (11 980), 480 (10 850), 494 (11 050), and 528 (6045) nm. Anal. ($C_{35}H_{35}F_3N_2O_{16}\cdot H_2O$) C, H, F, N.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetyl-5-glutamyl)adriamycin (17)**: UV–vis λ_{max} (ϵ) 232 (40 350), 250 (27 615), 290 (12 915), 480 (9670), 494 (9740), and 528 (5790) nm. Anal. ($C_{36}H_{37}F_3N_2O_{16}\cdot H_2O$) C, H, N; F: calcd, 6.88; found, 6.21.

***N*-(Trifluoroacetyl)-14-*O*-(*N*-acetylglutaminyl)adriamycin (18)**: UV–vis λ_{max} (ϵ) 233 (38 840), 250 (26 475), 286 (9765); 480 (11 440), 495 (11 515), and 529 (6925) nm; NMR δ 2.03 (3 H, $NHCOCH_3$), 6.13 (d, J = 8 Hz, 1 H, N*H*$COCH_3$). Anal. ($C_{36}H_{38}F_3N_3O_{15}\cdot H_2O$) C, H, F, N.

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