Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 227–232

BIOORGANIC & MEDICINAL CHEMISTRY

New Chemical and Biological Aspects of Artemisinin-Derived Trioxane Dimers

Gary H. Posner,[a,*] John Northrop,[a] Ik-Hyeon Paik,[a] Kristina Borstnik,[a] Patrick Dolan,[b] Thomas W. Kensler,[b] Suji Xie[c] and Theresa A. Shapiro[c]

[a]*Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, Baltimore, MD 21218, USA*
[b]*Division of Toxicological Sciences and The Environmental Health Sciences Center, Department of Environmental Health Sciences, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205, USA*
[c]*Division of Clinical Pharmacology, Department of Medicine, School of Medicine, The Johns Hopkins University, Baltimore, MD 21205, USA*

Received 11 June 2001; accepted 23 July 2001

Abstract—Joining two 10-deoxoartemisinin trioxane units via a *p*-diacetylbenzene linker produces new C-10 non-acetal dimers **3b** and **3c**. ^{1}H NMR spectroscopy allows unambiguous assignment of the stereochemistry at C-10 in these dimers. Successful replacement of both carbonyl oxygen atoms in these diketone dimers by fluorine atoms produces new tetrafluorinated dimers **5a** and **5b**. Each dimer was evaluated in vitro for antimalarial, antiproliferative, and antitumor activities; ketone dimers **3b** and **3c**, more than fluorinated dimers **5a** and **5b**, are promising for chemotherapy of both malaria and cancer.

Introduction

Some 1,2,4-trioxane dimers have high in vitro antimalarial, antiproliferative, and antitumor activities,[1–4] including in vivo anticancer activity.[5] We report here several significant chemical and biological advances in this area: (1) a new protocol (Scheme 1) for semi-synthesis of C-10 non-acetal dimers **3** starting with the natural 1,2,4-trioxane antimalarial aretmisinin (**1**) and proceeding via C-10 ester acetal **2**; (2) biological evaluation of new αβ-dimer **3b** and new αα-dimer **3c**; (3) replacement of the two carbonyl groups in ketone dimers **3** by pharmacologically beneficial fluorine atoms without destruction of the crucial trioxane pharmacophore unit; and (4) biological evaluation of tetrafluorinated trioxane dimers **5**.

Results and Discussion

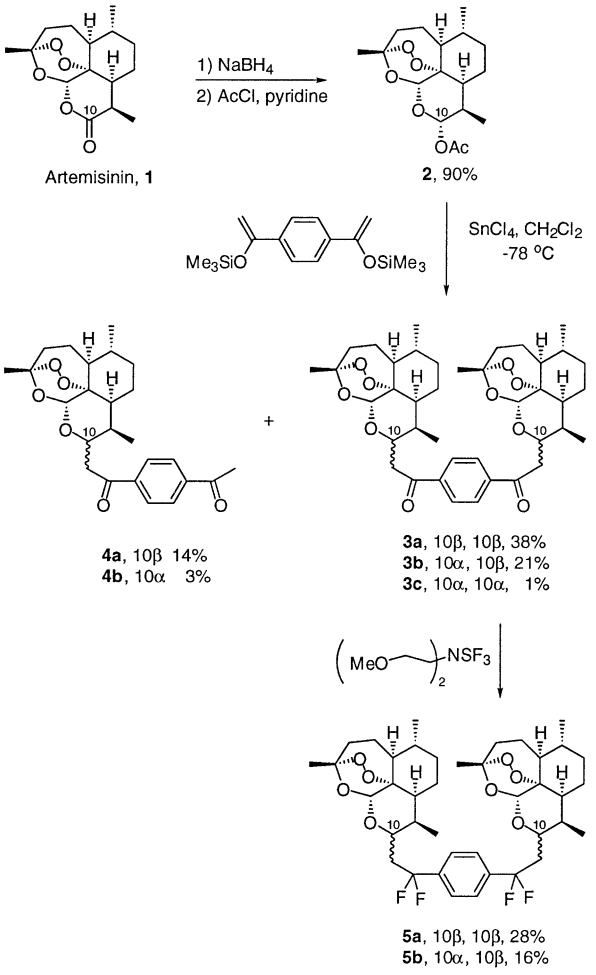
Synthesis of C-10 non-acetal dimer **3a** was achieved previously[5] in variable yield (maximum 26%) from artemether (the methyl ether of dihydroartemisinin) using titanium tetrachloride as promoter. As shown in Scheme 1, converting artemisinin (**1**) into dihydroartemisinin α-acetate (**2**)[6] in high yield then allowed mild tin tetrachloride-promoted double coupling with the bisenol trimethylsilyl ether of *p*-diacetylbenzene to produce not only previously reported[5] ββ-dimer **3a** now reproducibly and in improved 38% yield but also, for the first time, αβ-dimer **3b** (21% yield) and also αα-dimer **3c** (1% yield). Separation of these diastereomers was achieved chromatographically via preparative HPLC. C-10 stereochemistry was assigned by ^{1}H NMR spectroscopy as has been done previously in related C-10 substituted trioxanes.[7–9] In ββ-dimer **3a** and αα-dimer **3c**, the aromatic protons are equivalent, producing a singlet at δ 8.0, whereas in αβ-dimer **3b** the aromatic protons are non-equivalent, producing an AB quartet. Distinction between symmetrical ββ-dimer **3a** and symmetrical αα-dimer **3c** relies on several characteristic peaks that are at lower field in the ββ- vs αα-dimer (e.g., δ 5.32 vs 5.24; 5.08 vs 4.19; 2.79 vs 2.42; 0.96 vs 0.95; 0.90 vs 0.82). Also, the doublet of the AB quartet at δ 3.20 has a smaller coupling constant in the ββ-dimer **3a** than in the αα-dimer **3c**. Monomers **4** are characterized by a methyl singlet at δ 2.6. On analytical TLC plates using 20% EtOAc/hexanes, the R_f values are diacetylbenzene > monomers > dimers.

Introduction of one or more fluorine atoms into a biologically active compound often produces an analogue

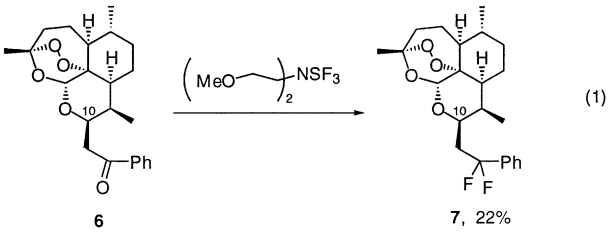
*Corresponding author. Tel.: +1-410-516-4670; fax: +1-410-516-8420; e-mail: ghp@jhunix.hcf.jhu.edu

0968-0896/02/$ - see front matter
PII: S0968-0896(01)00270-X

having more desirable pharmacological properties.[10,11] Fluorination of diketones **3** with diethylaminosulfur trifluoride (DAST), however, was unsuccessful. Fluorination at a higher temperature using the more robust, commercially available fluorinating agent bis(2-methoxyethyl)aminosulfur trifluoride (BAST) successfully produced tetrafluorinated dimers **5a** and **5b**, albeit in only modest yields. For comparison of the biological activity of fluorinated dimers **5** with that of a fluorinated monomer, BAST fluorination of 10β-benzoylmethyl-10-deoxoartemisinin (**6**) was performed to provide difluorinated monomer **7** [eq (1)]).[12]



Scheme 1.



Using our standard assay,[13] the in vitro antimalarial potencies of non-fluorinated dimers **3** and of fluorinated dimers **5** against chloroquine-sensitive *Plasmodium falciparum* (NF54) parasites were established (Table 1). Dicarbonyl dimers **3a**–**3c** are 2–5 times more potent than artemisinin (**1**), whereas tetrafluorinated dimers **5a** and **5b** are 2–4 times less potent than artemisinin (**1**). Also, both dicarbonyl dimers **3a** and **3b** are more potent than trioxane monomers **4** and **6**, and monomer **6** is comparable in potency to its difluorinated version **7**.

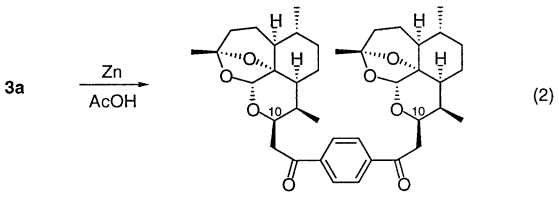
Table 1. Antimalarial activities in vitro

Compd	IC_{50} (nM)[a]
3a	1.9
3b	1.7
3c	3.9
4a	4.4
4b	3.0
5a	28
5b	15
6	5.2
7	5.1
Artemisinin	7.6±1.4

[a]Antimalarial activity was determined against the chloroquine-sensitive NF54 strain of *P. falciparum* as reported previously.[13] The standard deviation for each set of quadruplicates was an average of 11% ($\leq$ 58%) of the mean. R^2 values for the fitted curves were $\leq$ 0.982. Artemisinin activity is mean±standard deviation of concurrent control ($n=9$).

Although these trioxane dimers do not have enormously increased *antimalarial* potency over that of the corresponding trioxane monomers, these trioxane dimers (but not the monomers) do have potent *antiproliferative* properties. Antiproliferative activities, measured in vitro using murine keratinocytes as described previously,[14] are shown in Figure 1 for new non-fluorinated dimers **3b** and **3c** and for new fluorinated dimers **5a** and **5b**. It is noteworthy that these dimers are more effective at 1 μM concentration than calcitriol (1α,25-dihydroxyvitamin D_3), the hormonally active form of vitamin D_3 that is used clinically as a drug to treat psoriasis,[15] a skin disorder characterized by uncontrolled cell proliferation. Even at 0.1 μM concentrations, dimers **3b** and **3c** are still more antiproliferative than calcitriol. The fluorinated dimers **5** are no more potent in this assay than the non-fluorinated dimers **3**.

To test whether a corresponding dimer lacking the two peroxide units has any antiproliferative activity, trioxane dimer **3a** was doubly deoxygenated to form bisdeoxy dimer **3a′** [eq (2)]. Even at 1 μM concentration, however, bisdeoxy dimer **3a′** was not antiproliferative (data not shown). Dioxolanes (like this bisdeoxy compound **3a′**, antimalarial $IC_{50}=1030$ nM) are known to have weak or no antimalarial activity.[16] The structural requirement of two trioxane units in order for dimers like **3** to have both high antimalarial and high antiproliferative activities does not necessarily mean, however, that the same biological mechanism is operative in both cases. At this time, although the mechanistic details underlying a trioxane's antimalarial activity are becoming clear,[16,17] the biological mechanism of a trioxane dimer's antiproliferative action remains to be clarified.



(2)

Growth inhibitory activities at nanomolar to micromolar concentrations, measured in vitro as described previously using a diverse panel of 60 human cancer cell lines in the National Cancer Institute (NCI's) Developmental and Therapeutics Program,[18] indicate that our non-fluorinated dimers **3b** and **3c** are particularly inhibitory to leukemia cells, and these dimers are very selectively potent in a few other cancer cell lines (e.g., colon 205, ovarian cancer OVCAR-4, non-small cell lung EKVX, Fig. 2); in examining total growth inhibition (TGI), bars extending to the right of the centerline indicate cells more sensitive than average to a particular dimer, whereas bars to the left indicate less sensitive cells. Disappointingly, the fluorinated dimers **5** are less active in these assays than the non-fluorinated dimers **3**. The highly selective and powerful anticancer activities of dimers **3b** and **3c**, coupled with their lack of cytotoxicity, make these promising lead compounds for further preclinical study in dual action chemotherapy of both malaria and cancer.[19–21]

Experimental

Synthesis of dimers 3 and monomers 4

A flame dried 100 mL round bottom flask was charged with the dihydroartemisinin acetate **2** (1.60 g, 4.90 mmol) and the bisenol trimethylsilyl ether of *p*-diacetylbenzene (750 mg, 2.45 mmol) in CH_2Cl_2 (32 mL) then cooled to −78 °C (the optimal temperature for this coupling reaction). $SnCl_4$ (5.30 mL, 1.0 M in CH_2Cl_2) was added dropwise over 20 min via gas-tight syringe through a needle against which a piece of dry ice was held during the entire 20 min. TLC immediately after full addition of $SnCl_4$ showed the complete consumption of starting material and formation of a mixture of products. The reaction was quenched with satd. $NaHCO_3$ solution, then let warm to room temperature. The aqueous layer was extracted (3×) with CH_2Cl_2, the organic layer was combined, dried over $MgSO_4$, filtered and concentrated. Further purification by column chromatography (30% ethyl acetate/hexane) afforded a fraction containing an elimination product (147.0 mg, 0.552 mmol, 11%) and another fraction containing the ββ-dimer, αβ-dimer, αα-dimer, β-monomer, and α-monomer. This fraction was purified again by column chromatography [(100% CH_2Cl_2 pack; 5% ethyl acetate/CH_2Cl_2(monomer eluted); 15% ethyl acetate/CH_2Cl_2(dimer eluted)]. Finally the isomers of the dimers and monomers were separated by HPLC. Dimers: (silica preparative column 20% ethyl acetate/hexanes) ββ-dimer, **3a** (649 mg, 0.934 mmol, 38% R_t = 39.1 min); αβ-dimer, **3b** (361 mg, 0.520 mmol, 21% R_t = 37.1 min); αα-dimer, **3c** (20.1 mg, 0.029 mmol, 1.2%, R_t = 35.5 min). Monomers: (silica preparative column 95:4:1 hexanes/CH_2Cl_2/EtOH) β-monomer, **4a** (288 mg, 0.670 mmol, 14%, R_t = 62.2 min); α-monomer, **4b** (61.7 mg, 0.140 mmol, 3%, R_t = 55.8 min).

αβ-Dimer 3b. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (ABq, J_{AB} = 8.4 Hz, $\Delta\nu_{AB}$ = 30.9 Hz, 4H), 5.32 (s, 1H), 5.24 (s, 1H), 5.08 (m, 1H), 4.18 (m, 1H), 3.20 (dABq, J_d = 5.8 Hz, J_{AB} = 15.8 Hz, $\Delta\nu_{AB}$ = 153.8 Hz, 2H), 3.20

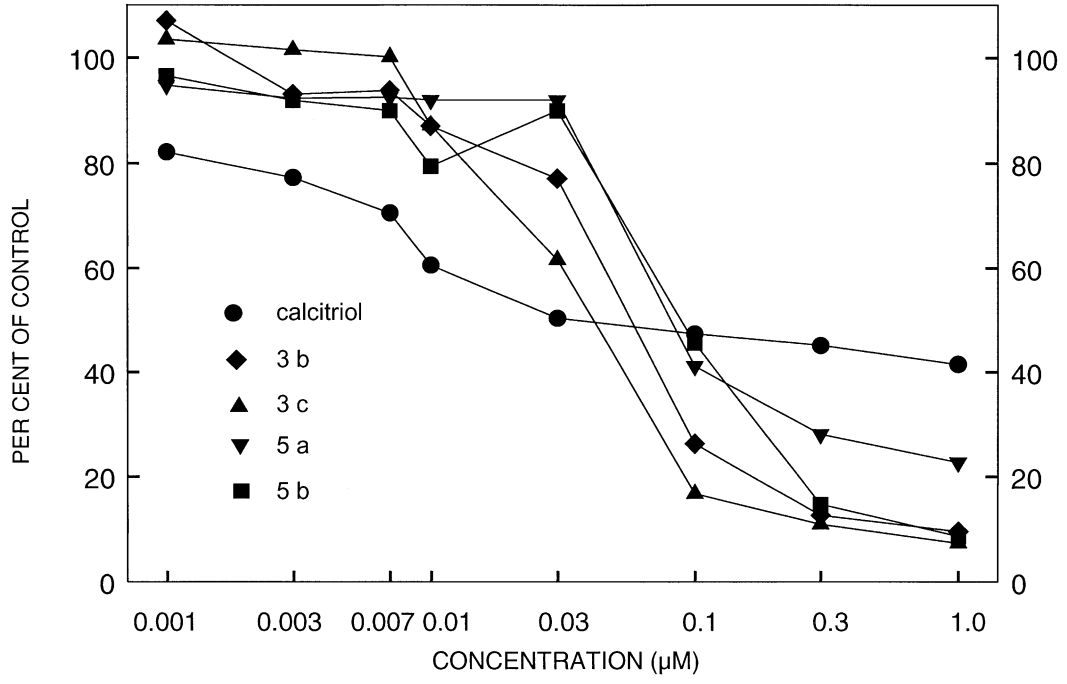


Figure 1. Dose–response effect of analogues on keratinocyte proliferation (96 h).

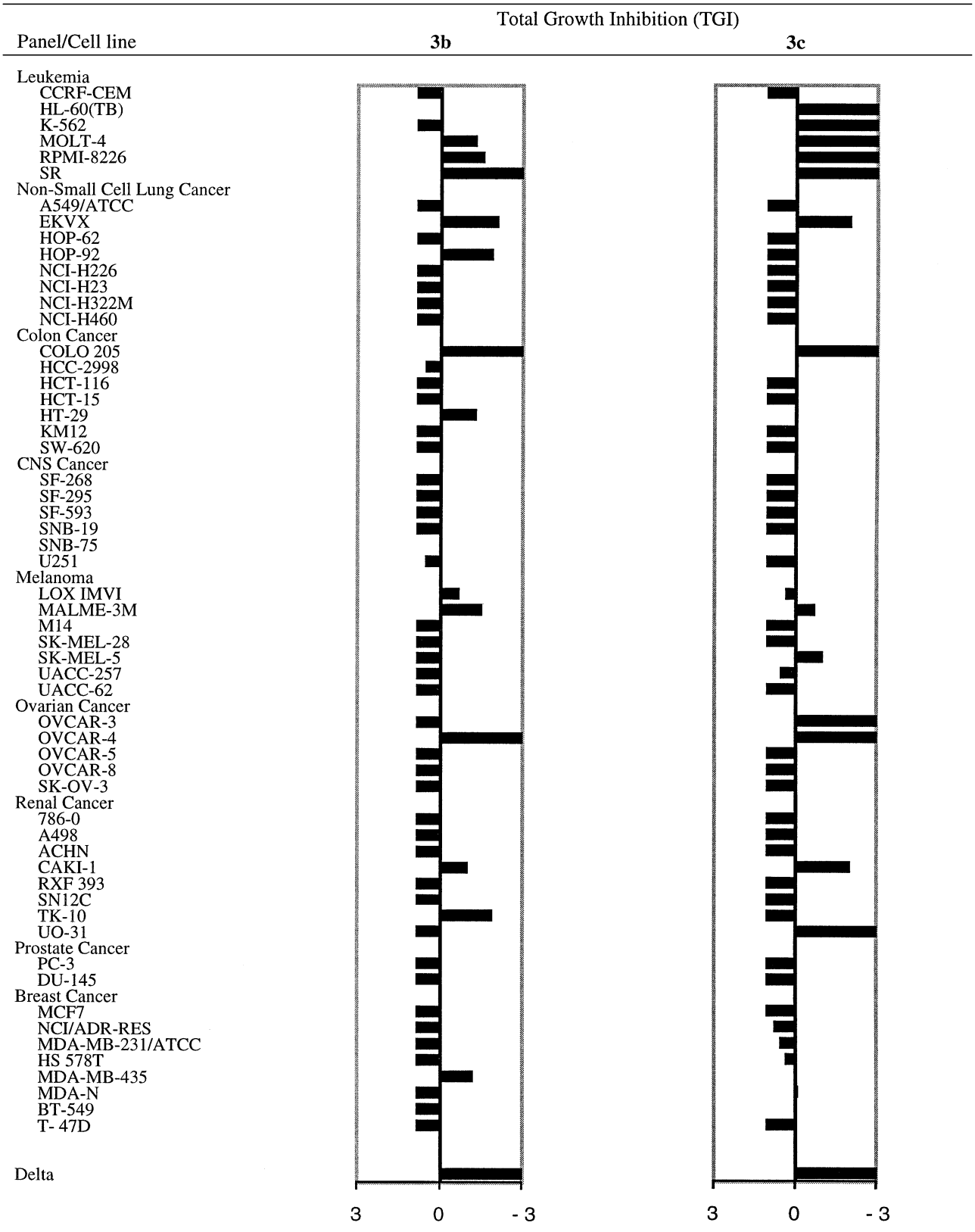

Total Growth Inhibition (TGI)
Panel/Cell line
3b
3c
Leukemia
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR
Non-Small Cell Lung Cancer
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
Colon Cancer
COLO 205
HCC-2998
HCT-116
HCT-15
HT-29
KM12
SW-620
CNS Cancer
SF-268
SF-295
SF-593
SNB-19
SNB-75
U251
Melanoma
LOX IMVI
MALME-3M
M14
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
Ovarian Cancer
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3
Renal Cancer
786-0
A498
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31
Prostate Cancer
PC-3
DU-145
Breast Cancer
MCF7
NCI/ADR-RES
MDA-MB-231/ATCC
HS 578T
MDA-MB-435
MDA-N
BT-549
T- 47D
Delta
3
0
- 3
3
0
- 3

Figure 2.

(dABq, $J_d = 6.6$ Hz, $J_{AB} = 16.0$ Hz, $\Delta\nu_{AB} = 84.4$ Hz, 2H), 2.79 (m, 1H), 2.42 (m, 1H), 2.32 (m, 2H), 2.2–1.4 (m, 20H), 1.33 (s, 3H), 1.30 (s, 3H), 0.97 (d, $J = 6.0$ Hz 3H), 0.95 (d, $J = 6.8$ Hz 3H), 0.89 (d, $J = 7.6$ Hz 3H), 0.82 (d, $J = 7.2$ Hz 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.8, 197.9, 140.9, 140.1, 129.0, 128.4, 104.2, 103.1, 92.0, 89.8, 81.0, 81.0, 71.7, 70.2, 52.2, 46.3, 44.2, 43.6, 40.7, 37.7, 37.5, 36.7, 36.5, 34.6, 34.3, 33.0, 30.1, 26.2, 26.0, 25.0, 24.9, 21.7, 20.5, 20.3, 14.2, 13.1; HRMS (ES) m/z calcd for $C_{40}H_{55}O_{10}$(M + H) 695.3795, found 695.3810; mp 124–127 °C.

αα-Dimer 3c. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 4H), 5.24 (s, 2H), 4.19 (ddd $J = 11.2$, 7.2, 4 Hz, 2H), 3.20 (dABq, $J_d = 5.8$ Hz, $J_{AB} = 15.8$ Hz, $\Delta\nu_{AB} = 153.8$ Hz, 4H), 2.42 (m, 2H), 2.32 (m, 2H), 2.02–1.96 (m, 2H), 1.90–1.87 (m, 2H), 1.75–1.70 (m, 4H), 1.59–1.43 (m, 4H), 1.35–1.20 (m, 3H), 1.34 (s, 6H), 1.10–1.00 (m, 3H), 0.98–0.83 (m, 2H), 0.95 (d, $J = 6.0$ Hz 6H), 0.82 (d, $J = 7.2$ Hz 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.7, 140.6, 128.5, 104.0, 91.8, 80.8, 71.5, 52.0, 46.1, 43.4, 37.3, 36.2, 34.1, 32.8, 26.0, 24.7, 21.4, 20.3, 14.0; IR ($CHCl_3$) 2926, 2874, 1685, 1455, 1378, 1265, 1206, 1132, 1090, 1061, 1044, 731 cm^{-1}; HRMS (ES) m/z calcd for $C_{40}H_{54}NaO_{10}$ (M + Na) 717.3615, found 717.3605; mp 107–108 °C.

β-Monomer 4a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 4H), 5.32 (s, 1H), 5.08 (m, 1H), 3.20 (dABq, $J_d = 6.8$ Hz, $J_{AB} = 16.0$ Hz, $\Delta\nu_{AB} = 87.3$ Hz, 2H), 2.78 (m, 1H), 2.63 (s, 3H), 2.29 (ddd,$J = 14.4$, 13.4, 3.8 Hz, 1H), 2.02–1.96 (m, 1H), 1.95–1.88 (m, 1H), 1.85–1.79 (m, 1H), 1.74–1.65 (m, 2H), 1.50–1.20 (m, 5H), 1.28 (s, 3H), 0.96 (d, $J = 6.0$ Hz 3H), 0.90 (d, $J = 7.2$ Hz 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 197.7, 140.4, 140.2, 128.7, 103.1, 89.8, 81.0, 70.2, 52.2, 44.2, 40.8, 37.7, 36.7, 34.6, 30.1, 27.1, 25.9, 25.0, 20.3, 13.1; HRMS (ES) m/z calcd for $C_{25}H_{33}O_6$ (M + H) 429.2277, found 429.2286; mp 51-52 °C.

α-Monomer 4b. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (tABq, $J_t = 2.0$ Hz, $J_{AB} = 8.6$ Hz, $\Delta\nu_{AB} = 32.7$ Hz, 4H), 5.23 (s, 1H), 4.17 (m, 1H), 3.19 (dABq, $J_d = 5.8$ Hz, $J_{AB} = 15.6$ Hz, $\Delta\nu_{AB} = 162.40$ Hz, 2H), 2.62 (s, 3H), 2.42 (m, 1H), 2.33 (ddd,$J = 14.4$, 13.6, 4.0 Hz, 1H), 2.00–1.94 (m, 1H), 1.88–1.81 (m, 1H), 1.74–1.69 (m, 3H), 1.53–1.40 (m, 3H), 1.31 (s, 3H), 1.32–1.19 (m, 2H), 0.94 (d, $J = 6.0$ Hz 3H), 0.82 (d, $J = 7.2$ Hz 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.9, 197.8, 141.1, 140.1, 129.0, 128.4, 104.2, 92.0, 80.9,71.8, 52.1, 46.3, 43.5, 37.5, 36.4, 34.3, 32.9, 27.1, 26.1, 24.9, 21.6, 20.5, 14.2; HRMS (ES) m/z calcd for $C_{25}H_{33}O_6$ (M + H) 429.2277, found 429.2294; mp 56-57 °C.

Fluorination of dimers: general procedure

To a 7 mL Teflon vial (purchased from Berghof/America Inc., Coral Springs, FL, USA) the dicarbonyl dimer (0.050 mmol) and BAST (bis[2-methoxyethyl]amino sulfur trifluoride) (0.40 mL, 2.2 mmol, 43 equiv, purchased from SynQuest Lab. Inc. (Alachua, FL, USA) and distilled prior to use) were added and the vial capped with a Teflon cap and sealed with Teflon tape. The resulting mixture was stirred at 80 °C for 24 h. The reaction mixture was diluted with 40 mL dichloromethane (EM Science, Gibbstown, NJ, USA) and quenched with 40 mL ice-cold satd $NaHCO_3$ solution. The organic layer was separated and washed with 40 mL brine, dried over $MgSO_4$, filtered and concentrated to give the fluorinated product as a yellow oil.

Tetrafluorinated ββ-dimer 5a. The crude mixture was purified by column chromatography (silicagel, 14% ethyl acetate/hexanes). Further purification by HPLC (silica semi-preparative column, 12% ethyl acetate/hexanes, $R_t = 16.4$ min) provided **5a** (10.4 mg, 0.014 mmol, 28%) as a white solid: mp 148–150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 4H), 5.26 (s, 2H), 4.49–4.45 (m, 2H), 2.73–2.68 (m, 2H), 2.57–2.44 (m, 2H), 2.35–2.16 (m, 4H), 1.93–1.87 (m, 2H), 1.82–1.76 (m, 2H), 1.68–1.63 (m, 2H), 1.61–1.50 (m, 3H), 1.48–1.18 (m, 9H), 1.39 (s, 6H), 1.05–0.90 (m, 2H), 0.95 (d, $J = 6.4$ Hz 6H), 0.83 (d, $J = 7.6$ Hz 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5 (t, $J = 26.6$ Hz), 125.4 (t, $J = 6.1$ Hz), 122.2 (t, $J = 243$ Hz), 103.3, 88.7, 80.7, 69.5, 52.3, 44.3, 39.0 (t, $J = 26.6$ Hz), 37.5, 36.5, 34.4, 30.0, 26.0, 24.6, 24.6, 20.2, 13.1; ^{19}F NMR ($CDCl_3$, $CFCl_3$ as a reference) δ −90.0 (ddd, $J = 248$, 16.9, 13.0 Hz) −95.4 (ddd, $J = 248$, 16.9, 16.9 Hz); IR ($CHCl_3$) 2940, 2876, 1453, 1409, 1377, 1322, 1096, 1060, 1013, 876, 846, 732 cm^{-1}; HRMS (ES) m/z calcd for $C_{40}H_{54}F_4NaO_8$ (M + Na) 761.3653, found 761.3643.

Tetrafluorinated αβ-dimer 5b. The crude mixture was purified by column chromatography (silicagel, 14% ethyl acetate/hexanes). Further purification by HPLC (silica semi-preparative column, 10% ethyl acetate/hexanes, $R_{t=}21.0$ min) provided **5b** (6.0 mg, 0.008 mmol, 16%) as a white solid: mp 146–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, $J = 0.8$ Hz 4H), 5.27 (s, 1H), 5.08 (s, 1H), 4.49–4.45 (m, 1H), 3.68–3.62 (m, 1H), 2.71–2.67 (m, 1H), 2.52–2.40 (m, 2H), 2.37–2.18 (m, 5H), 2.05–1.76 (m, 5H), 1.70–1.61 (m, 3H), 1.51–1.16 (m, 10H), 1.39 (s, 3H), 1.31 (s, 3H), 1.04–0.87 (m, 2H), 0.95 (d, $J = 6.0$ Hz 3H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.81 (d, $J = 7.6$ Hz, 3H), 0.80 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.6 (t, $J = 26.6$ Hz), 140.4 (t, $J = 26.6$ Hz), 125.4 (t, $J = 6.1$ Hz), 125.2 (t, $J = 6.1$ Hz), 122.2 (t, $J = 243$ Hz), 122.0 (t, $J = 243$ Hz), 103.9, 103.3, 91,6, 88.8, 80.7, 80.5, 69.6, 69.5, 52.3, 51.9, 45.9, 44.3, 42.5 (t, $J = 26.6$ Hz), 39.0 (t, $J = 26.6$ Hz), 37.5, 37.3, 36.5, 36.2, 34.4, 34.1, 32.2, 30.0, 26.0, 25.9, 24.7, 24.7, 24.5, 21.3, 20.3, 20.2, 14.0, 13.1; ^{19}F NMR ($CDCl_3$, $CFCl_3$ as a reference) δ −90.0 (ddd, $J = 252$, 17.7, 14.5 Hz, 2F),-93.2 (ddd, $J = 247$, 16.7, 11.3 Hz, 2F), −95.8 to −96.5 (m, 4F); HRMS (ES) m/z calcd for $C_{40}H_{54}F_4NaO_8$ (M + Na) 761.3653, found 761.3645.

Difluorinated monomer 7

BAST fluorination of ketone monomer **6** gave a crude product mixture that was purified by column chromatography (silicagel, 10% ethyl acetate/hexanes). Further purification by HPLC (silica semi-preparative column, 5% ethyl acetate/hexanes, $R_t = 24.3$ min) provided difluorinated monomer **7** (4.5 mg, 0.011 mmol, 22%) as a white solid: mp 134–135 °C; ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.53–7.51 (m, 2H), 7.42–7.41 (m, 3H), 4.50 (ddd, $J=8.6$, 6.0, 2.8 Hz, 1H), 2.75–2.66 (m, 1H), 2.56–2.43 (m, 1H), 2.35–2.27 (m, 1H), 2.28–2.19 (m, 1H), 2.02 (ddd, $J=14.4$, 4.8, 2.8 Hz, 1H), 1.93–1.86 (m, 1H), 1.81–1.75 (m, 1H), 1.68–1.62 (m, 1H), 1.61–1.55 (m, 1H), 1.49–1.41 (m, 1H), 1.39 (s, 3H), 1.32–1.19 (m, 4H), 1.00–0.87 (m, 1H), 0.95 (d, $J=6.0$ Hz, 3H), 0.82 (d, $J=7.6$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.1 (t, $J=28.0$ Hz), 129.6, 128.3, 125.2 (t, $J=6.5$ Hz), 122.4 (t, $J=243$ Hz), 103.3, 88.8, 80.7, 69.6 (t, $J=6.0$ Hz), 52.3, 44.4, 39.0 (t, $J=27.0$ Hz), 37.5, 36.5, 34.4, 30.0, 26.0, 24.7, 24.5, 20.2, 13.1; ^{19}F NMR ($CDCl_3$, $CFCl_3$ as a reference) δ 93.2 (ddd, $J=247$, 18.0, 11.0 Hz), −95.8 (ddd, $J=247$, 17.6, 17.6 Hz); IR ($CHCl_3$) 3010, 2921, 2973, 1490, 1377, 1321, 1260, 1098, 1056, 1016, 876, 802, 699 cm^{-1}; HRMS (ES) m/z calcd for $C_{23}H_{30}F_2NaO_4$ (M + Na) 431.2004, found 431.2010.

ββ- Bisdeoxydimer 3a′. Immediately prior to use, Zn dust was washed with 5% HCl (3×), de-ionized water (3×), EtOH (3×), diethyl ether (3×), then dried in vacuo and placed under Ar. A flamed dried 25 mL round bottomed flask was charged with the ββ-dimer **3a** (35.0 mg, 0.050 mmol), glacial acetic acid (15.0 mL, 26.0 mmol), and washed Zn dust (8.2 mg, 0.13 mmol). The reaction was monitored by TLC and during 8 h additional Zn dust (25 mg, 0.40 mmol) was added. The reaction was transferred with $CHCl_3$ to a large flask through a glass frit to remove the Zn dust. The solution was slowly neutralized with satd $NaHCO_3$ until evolution of gas ceased. The aqueous layer was extracted with $CHCl_3$, the organic layers combined, dried over $MgSO_4$, and concentrated. Purification by column chromatography (silicagel, 0–25% ethyl acetate/petroleum ether) afforded a white powder that was a mixture of the ββ-bisdeoxydimer **3a′** and starting material **3a**. Further purification by HPLC (silica preparative column; 95:4:1 hexanes CH_2Cl_2 EtOH) provided ββ-bisdeoxydimer **3a′** (28.1 mg, 0.042 mmol, 85%, $R_t=18.7$ min) as a white solid: mp 185–186 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 4H), 5.21 (s, 2H), 4.78 (q $J=7.2$ Hz, 2H), 3.14 (dABq, $J_d=7.0$ Hz, $J_{AB}=16.2$ Hz, $\Delta\nu_{AB}=41.8$ Hz, 4H), 2.46 (m, 2H), 1.98 (m, 2H), 1.86–1.76 (m, 6H), 1.72–1.54 (m, 10H), 1.44 (s, 6H), 1.30–1.16 (m, 4H), 0.88 (d, $J=7.2$ Hz, 6H), 0.86 (d, $J=9.2$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.5, 140.3, 128.5, 107.4, 97.2, 82.6, 65.9, 45.4, 41.8, 40.5, 35.8, 34.7, 34.6, 29.2, 25.4, 23.7, 22.4, 19.0, 12.9; IR (neat) 2952, 2874, 1688, 1384, 1208, 1097, 1000 cm^{-1}; HRMS (ES) m/z calcd for $C_{40}H_{55}O_8$ (M + H) 663.3897, found 663.3874.

Acknowledgements

We thank the NIH (AI-34885, CA-44530, and RR-00052) and the Burroughs Wellcome Fund for generous financial support, the NCI's Developmental and Therapeutics program for evaluation of these dimers, the Pfizer Pharmaceutical Co. for a summer research fellowship to J.N., and Dr. Paul O'Neill (Liverpool, UK) for helpful suggestions.

References and Notes

1. Beekman, A. C.; Barenstsen, A. R. W.; Woerdenbag, H. J.; Van Uden, W.; Pras, N. *J. Nat. Prod.* **1997**, *60*, 325.
2. Galal, A. M.; Ahmad, M. S.; El-Feraly, F. S. *J. Nat. Prod* **1996**, *59*, 917.
3. Venugopalan, B.; Bapat, C. P.; Karnik, P. J.; Catterjee, D. K.; Iyer, N.; Lepcha, D. *J. Med. Chem.* **1995**, *38*, 1922.
4. Posner, G. H.; Ploypradith, P.; Hapangama, W.; Wang, D.; Cumming, J. N.; Klinedinst, D.; Shapiro, T. A.; Dolan, P.; Kensler, T. W.; Daughenbaugh, R.; Murray, C. K.; Zheng, Q. Y. *Bioorg. Med. Chem.* **1997**, *5*, 1257.
5. Posner, G. H.; Ploypradith, P.; Parker, M. H.; O'Dowd, H.; Woo, S. H.; Northrop, J.; Krasavin, M.; Dolan, P.; Kensler, T. W.; Xie, S.; Shapiro, T. A. *J. Med. Chem.* **1999**, *42*, 4275.
6. Ying, L.; Peilin, Y.; Yixin, C.; Liangquan, L.; Yuanzhu, G.; Desheng, W.; Yaping, Z. *Acta Pharm. Sinica* **1981**, *26*, 429.
7. Ma, J.; Katz, E.; Ziffer, H. *Tetrahedron Lett.* **1999**, *40*, 8543.
8. Woo, S. H.; Parker, M. H.; Ploypradith, P.; Northrop, J.; Posner, G. H. *Tetrahedron Lett.* **1998**, *39*, 1533.
9. Jung, M.; Lee, S. *Heterocycles* **1997**, *45*, 1055.
10. Banks, R. E.; Smart, B. E.; Tatlow, J. C. *Organofluorine Chemistry: Principles and Commercial Applications*; Plenum: New York, 1994.
11. Park, B. K.; Kitteringham, N. R.; O'Neill, P. M. *Annu. Rev. Pharm. Tox.* **2001**, *41*, 443.
12. Related difluorinated trioxanes have recently been prepared using tin tetrachloride-promoted coupling: Chorki, F., Crouse, B.; Bonnet-Delpon, D.; Begue, J.-P.; Brigand, T.; Portella, C. *Tetrahedron Lett.* **2001**, *42*, 1487
13. For experimental details of the testing protocol, including references to development of the assay, see: Posner, G. H; González, L.; Cumming, J. N.; Klinedinst, D.; Shapiro, T. A. *Tetrahedron* **1997**, *53*, 37.
14. Posner, G. H.; Nelson, T. D.; Guyton, K. Z.; Kensler, T. W. J. *Med. Chem.* **1992**, *35*, 3280.
15. Holick, M. F. *Vitamin D: Molecular Biology, Physiology, and Clinical Applications*; Humana: Totowa, 1999.
16. Cumming, J. N.; Ploypradith, P.; Posner, G. H. *Adv. Pharmacol.* **1997**, *37*, 253.
17. Posner, G. H., Cumming, J. N.; Krasvin, M. In *Biomedical Chemistry: Applying Chemical Principles to the Understanding and Treatment of Disease*. Torrence, P., Ed., John Wiley, 2000, p 284.
18. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
19. Lai, H.; Singh, N. P. *Cancer Lett.* **1995**, *91*, 41.
20. Singh, N.P.; Lai, H. *Life Sciences*, in press
21. Efferth, T.; Dunstan, H.; Sauerbrey, A.; Miyachi, H.; Chitambar, C. R. *Int. J. Oncol.* **2001**, *18*, 767.