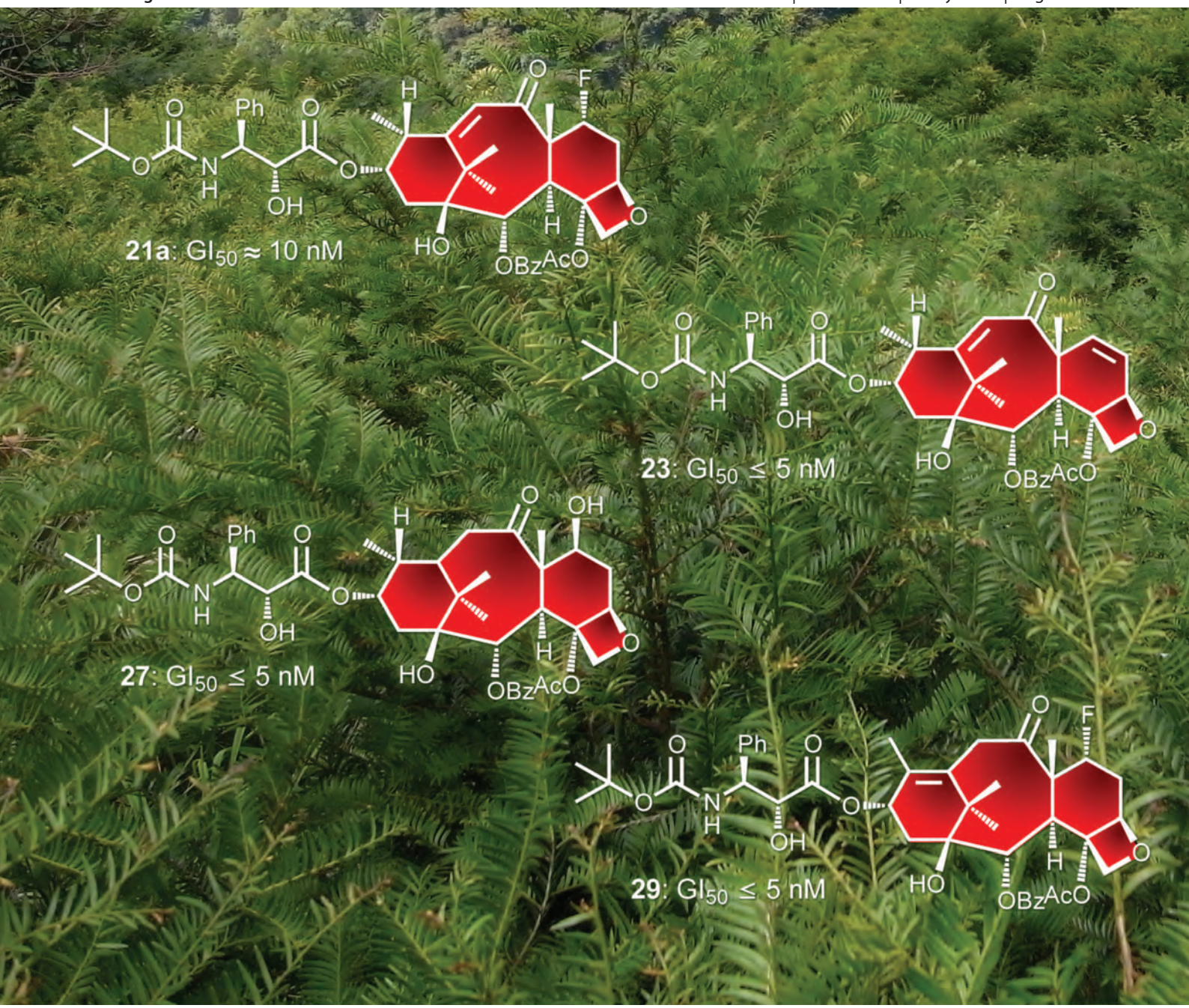
Organic & Biomolecular Chemistry

www.rsc.org/obc

Volume 11 | Number 25 | 7 July 2013 | Pages 4125–4272



ISSN 1477-0520

RSC Publishing

PAPER

Kyriacos C. Nicolaou and Roman A. Valiulin

Synthesis and biological evaluation of new paclitaxel analogs and discovery of potent antitumor agents

1477-0520(2013)11:25;1-8

Organic & Biomolecular Chemistry

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Synthesis and biological evaluation of new paclitaxel analogs and discovery of potent antitumor agents†‡

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 4154

Kyriacos C. Nicolaou*[a,b,c,d] and Roman A. Valiulin[a]

Received 2nd April 2013,
Accepted 3rd May 2013

DOI: 10.1039/c3ob40654g

www.rsc.org/obc

Reaction of 10-deacetylbaccatin III (**III**) and its 7-TES derivative (**IV**) with DAST under various conditions resulted in the formation of an array of new fluorinated and non-fluorinated 13-keto taxoid compounds (**2a–4a**) through a vinylogous pinacol–pinacolone rearrangement. Further fluorination of some of these products (**2a**, **3a**) with NFSi or Selectfluor gave additional derivatives. Sodium borohydride reduction of the 13-keto group of these products (**2a**, **2b**, **3a**, **3b**, **4a**, **8**, **9**, **11–14**) led to a series of 9α-hydroxy taxoid derivatives, which were esterified using the docetaxel side chain employing the corresponding protected β-lactam, followed by deprotection to furnish a library of docetaxel analogs and related compounds. A selected number of synthesized compounds (**7**, **10**, **19a**, **19b**, **21a**, **21b**, **23**, **27**, **29**, **34–36**) were submitted to the National Cancer Institute (NCI) 60 cell line screening program and tested for cytotoxic properties. Taxoids **19a**, **19b**, **21a**, **21b**, **23**, **27**, **29**, **34** and **35** were found to exhibit significant anticancer activity against various cancerous cell lines with **23**, **27**, and **29** being the most potent compounds, demonstrating GI_{50} values of ≤5 nM in several assays.

Introduction

Cancer remains as one of the most devastating diseases of our times with millions of patients suffering from it around the world.[1–6] Moreover, the disease is on the rise and no organ of the human body is immune to its intrusion. Despite the plethora of anticancer drugs currently available for combating cancer, serious deficiencies still prevail, particularly with regards to their efficacy and safety. These chemotherapeutic agents range from small organic molecules and natural products and their analogs, to antibodies and antibody–drug conjugates.[7,8]

Among the natural products and their semi-synthetic analogs, paclitaxel (**I**, Fig. 1) and docetaxel (**II**, Fig. 1) are perhaps the most well-known and frequently employed due to their potency, selectivity and novel mechanism of action.[9–11] Paclitaxel is clinically used in the treatment of breast cancer, advanced ovarian cancer, non-small cell lung cancer, and advanced forms of Kaposi's sarcoma,[12] whereas docetaxel is employed against breast cancer, non-small cell lung cancer, gastric cancer, prostate cancer, and squamous cell carcinoma of the head and neck.[13–15] Cabazitaxel (Jevtana, **II′**), a recently

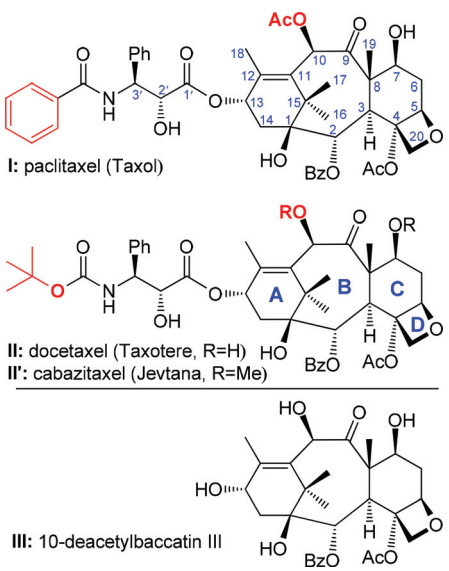


Fig. 1 Structures of paclitaxel (**I**), docetaxel (**II**), cabazitaxel (**II′**), and 10-deacetylbaccatin III (**III**). Bz = benzoyl; Ac = acetyl; Ph = phenyl.

[a]*Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA. E-mail: kcn@scripps.edu, kcn@rice.edu*
[b]*Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA*
[c]*Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA*
[d]*Department of Chemistry, Rice University, BioScience Research Collaborative, 6100 Main Street, Houston, Texas 77005, USA*

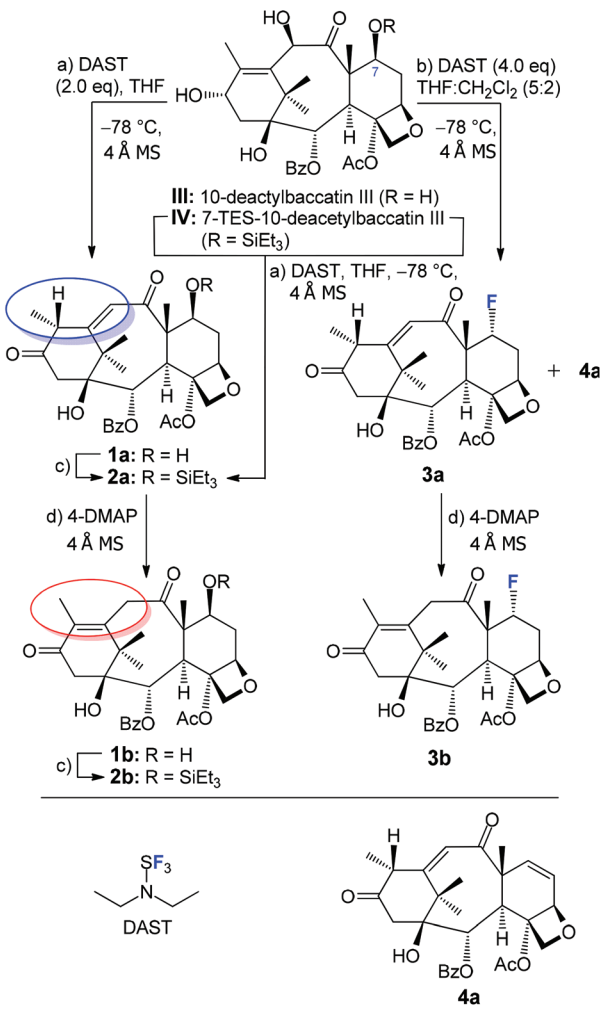
†Electronic supplementary information (ESI) available: Includes experimental procedures, selected physical data for the synthesized compounds, and further details of biological assays. CCDC 931453–931456. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob40654g

‡Dedicated to Professor Teruaki Mukaiyama on the occasion of his 85th birthday.

introduced derivative of docetaxel (docetaxel methylated at C7 and C10 hydroxyl groups), is used to treat hormone-refractory prostate cancer. The latter success underscores the importance of fine-tuning the structures of these agents in order to amplify their selectivity towards specific cancers, a theme that is currently attracting special attention in view of personalized medicine.

A large number of paclitaxel and docetaxel analogs became available over the last three decades leading to a useful body of structure–activity relationships (SARs).[16–38] Enabled by synthesis, these studies were facilitated by the ready availability of paclitaxel and its precursor 10-deacetylbaccatin III (**III**, Fig. 1). Motivated by the approval of cabazitaxel and the successes in medicinal chemistry resulting from the introduction of fluorine residues into small molecules,[39] we initiated a research program directed towards the synthesis of fluorinated and other novel taxoids starting from 10-deacetylbaccatin III (**III**, Scheme 1) and its 7-OTES derivative (**IV**, Scheme 1). The virtues of introducing fluorine atoms into organic molecules have been widely recognized and reported;[39–44] they include increased metabolic stability (resistance to biochemical degradation), enhanced lipophilicity (log*P*), and stronger binding affinities, often leading to improved pharmacological properties. It is notable that the current number of clinically used drugs containing one or more fluorine atoms stands at 20–30% and is steadily increasing.[40,42]

In this article, we report the results of our investigations on the synthesis and biological evaluation of new taxoids, including a number of fluorinated docetaxel analogs.[45]



Scheme 1 Reaction of 10-deacetylbaccatin III (**III**) with DAST. Synthesis of compounds **1a–4a** and **1b–3b**. Reagents and conditions: (a) DAST (2.0 equiv.), THF, 4 Å MS, –78 °C, 3 h, **III** → **1a** (93%); **IV** → **2a** (70%); (b) DAST (4.0 equiv.), THF–CH_2Cl_2 (5 : 2), 4 Å MS, –78→ 25 °C, 24 h, **III** → **3a** (63%), plus **4a** (≤20%); (c) Et_3SiCl (5.0 equiv.), Et_3N (5.0 equiv.), 4-DMAP (0.5 equiv.), THF, 0 °C, 1.5 h, **1a** → **2a** (74%); **1b** → **2b** (80%); (d) 4-DMAP (2.5 equiv.), THF, 4 Å MS, 25 °C, 20–24 h, **1a** → **1b** (98%); **2a** → **2b** (96%); **3a** → **3b** (78%). DAST = (diethylamino)sulfurtrifluoride; TES = triethylsilyl; MS = molecular sieves; THF = tetrahydrofuran; 4-DMAP = 4-(dimethylamino)pyridine.

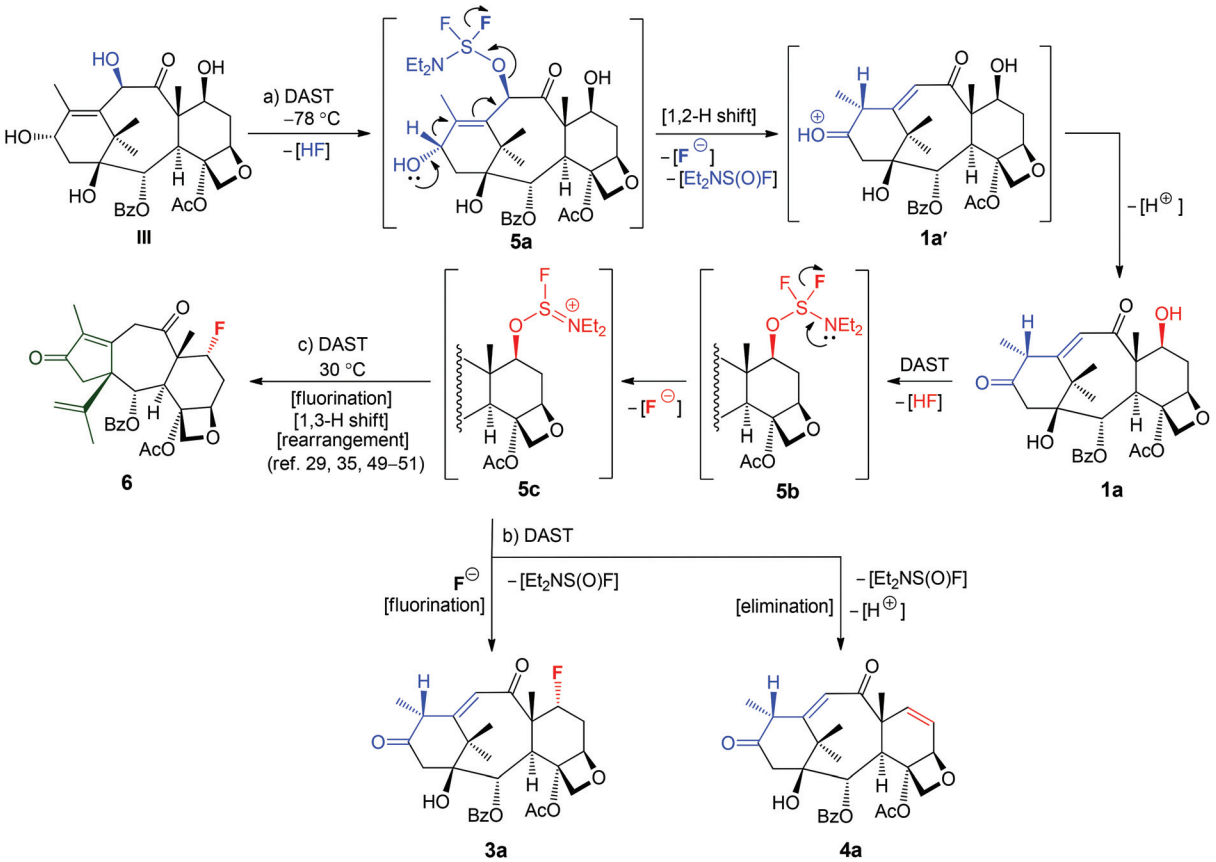
Results and discussion

Reaction of 10-deacetylbaccatin III (III) with DAST

The present study began with 10-deacetylbaccatin III (**III**) and its reaction with the common fluorinating agent (diethylamino)sulfur trifluoride (DAST).[46] Our intention was to obtain as many fluorinated species as possible for the purposes of converting them, through side chain attachment, to full docetaxel structures for biological evaluation. The top priority at the initial stage was, therefore, structural elucidation of products rather than yield optimization, the latter being postponed until the properties of the molecule would dictate it.

Thus, and as shown in Scheme 1, reaction of **III** with 2.0 equiv. of DAST in THF in the presence of 4 Å MS at −78 °C over 3 h [conditions (a), Scheme 1] furnished enone **1a** in 93% yield.[47] Product **1a** was found to be labile and easily transformed to its apparently more stable isomeric paclitaxel-related form **1b** (red ellipsoid, Scheme 1) on silica gel. This conversion could also be induced by 4-DMAP in THF at ambient temperature in 98% yield [conditions (d)]. For the purposes of further manipulations, compound **1a** was also converted to its 7-OTES derivative **2a** under conditions (c) [Et_3SiCl (5.0 equiv.), Et_3N (5.0 equiv.), 4-DMAP (0.5 equiv.), THF, 0 °C, 1.5 h; in 74% yield] and thence to the isomerized taxoid **2b** under basic conditions [conditions (d), 96% yield]. The latter was also obtained from the previously prepared taxoid **1b** upon silylation in 80% yield [conditions (c)]. Importantly, enone **2a** could also be prepared directly from 7-trimethylsilyl-10-deacetylbaccatin III[48] (**IV**) (Scheme 1) in 70% yield by exposure of the latter to DAST [2.0–4.0 equiv., THF, 4 Å MS, −78 °C, 1–3 h, conditions (a)].

Interestingly, 10-deacetylbaccatin III (**III**) reacted differently with DAST under modified conditions (b) (Scheme 1). Thus, treatment of **III** with 4.0 equiv. of DAST in THF–CH_2Cl_2 (5 : 2), in the presence of 4 Å MS, at −78 to 25 °C, over 24 h led to fluorinated enone **3a** in 63% yield accompanied by varying amounts (≤20%) of elimination product **4a**, inseparable by silica gel chromatography. The formation of product **4a** could be suppressed by diluting the reaction mixture with further



Scheme 2 Preparation of compounds **3a**, **4a**, and **6** and proposed mechanism for their formation. Reagents and conditions: see Scheme 1 for (a) and (b).

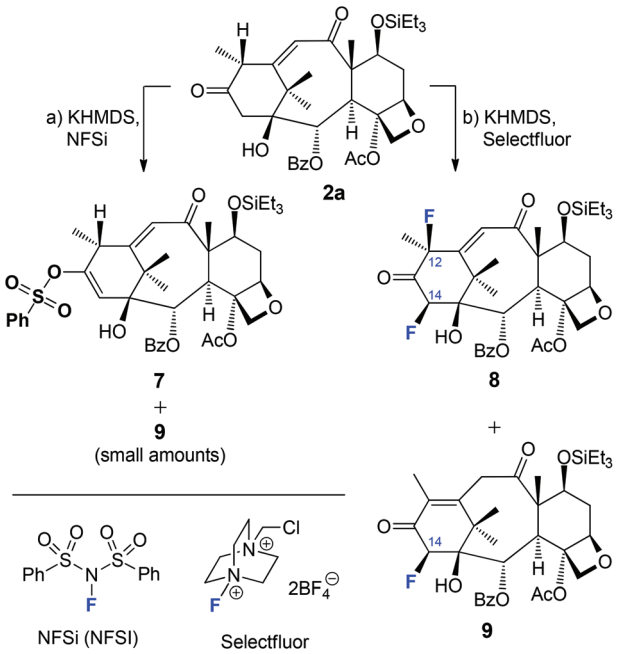
amounts of CH_2Cl_2 after the addition of the first 2.0 equiv. of DAST and carefully controlling the temperature during the reaction (see ESI† for experimental details). Similarly to **1a**, enone **3a** isomerizes to taxoid **3b** in 78% yield under conditions (d) (Scheme 1).

Plausible mechanistic explanations for the reactions mentioned in Scheme 1 are summarized in Scheme 2. Thus, initial reaction of **III** with DAST may produce intermediate **5a** with elimination of HF. This intermediate may then undergo concerted 1,2-*H*-shift and elimination of $Et_2NS(O)F$ and F^- to afford protonated species **1a′**, from which observed product **1a** may be formed through the loss of an H^+. A stepwise process involving the formation of an allylic carbocation for this vinylogous pinacol–pinacolone rearrangement (**5a** → **1a**) may also be envisioned. Reaction of hydroxyenone **1a** with further amounts of DAST may then lead, initially to **5b**, and thence to intermediate **5c**, from which all observed products (*i.e.* **3a**, **4a**, and **6**) may be generated[49–52] as shown in Scheme 2.

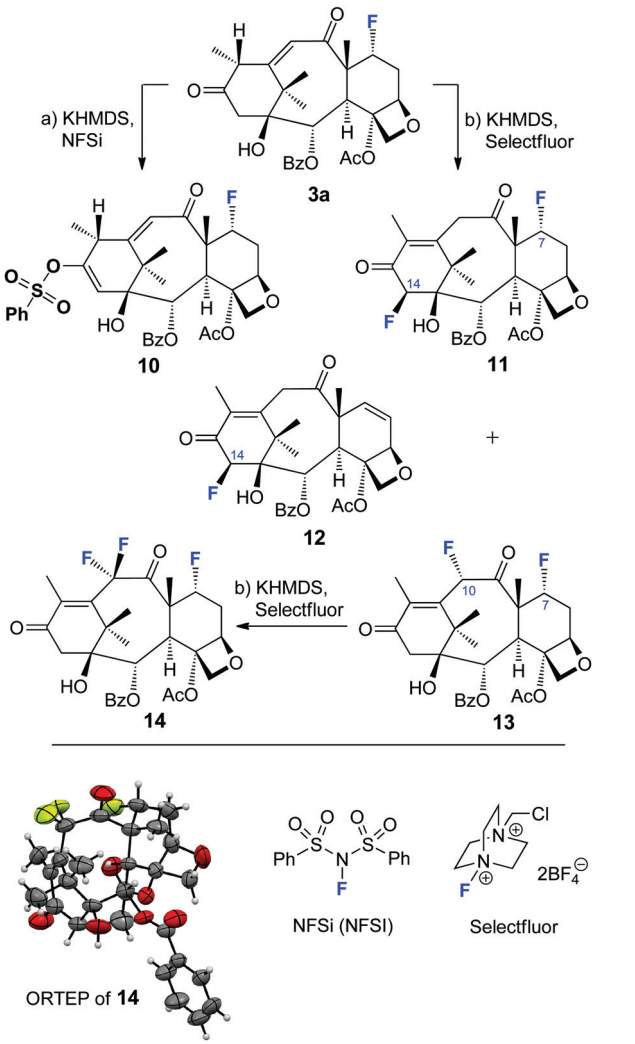
Reaction of enones 2a and 3a with NFSi and Selectfluor

Having gained access to the new taxoid scaffolds mentioned above, we proceeded to explore their chemistry with additional fluorinating agents. Enones **2a** and **3a** proved to be the most fertile in these pursuits as shown in Schemes 3 and 4, respectively. Thus, treatment of compound **2a** with *N*-fluoro-*N*-(phenylsulfonyl)benzenesulfonimide (NFSi)[53–56] and KHMDS in THF in the presence of 4 Å MS at −78 °C [conditions (a), Scheme 3] led to a complex mixture of products from which rather unexpected sulfonate **7** was isolated as a major product (33% yield), while the expected 14β-fluorotaxoid **9** was obtained as a minor product (≤5% yield). In contrast to these results, the reaction of **2a** with 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (F-TEDA-BF_4, or Selectfluor)[57] under conditions (b) (Scheme 3) [KHMDS (2.0 equiv.), Selectfluor (10.0 equiv.), THF–DMF (10 : 1), 4 Å MS, −78 °C, 1–2 h and 25 °C, 1 h] furnished 12β,14β-bis-fluorinated product **8** (24% yield) and varying amounts of 14β-fluorinated compound **9** (≤20%) depending on stoichiometry and reaction time.[58] The latter product is presumed to arise from **2a** *via* mono-fluorination and enone transposition.

7α-Fluoroenone **3a** also entered a number of interesting reactions with NFSi and Selectfluor (Scheme 4). Thus, treatment of **3a** with NFSi under conditions (a) [KHMDS (2.2 equiv.), NFSi (3.0 equiv.), THF, 4 Å MS, −78 °C, 1 h and 25 °C, 1 h] furnished 7α-fluoro vinyl sulfonate **10** (24% yield) mirroring the reaction of its 7-OTES counterpart **2a** (Scheme 3). On the other hand, reaction of **3a** with Selectfluor under conditions (b) [KHMDS (2.0 equiv.), Selectfluor (10.0 equiv.), THF–DMF (10 : 1), 4 Å MS, −78 °C, 1–2 h and 25 °C, 1 h] led to 7α,14β-difluoroenone **11** (≤5% yield) as a minor product (plus trace amounts of compound **12** (≤2%), presumably arising from contaminant **4a** present in starting material **3a**, see



Scheme 3 Fluorination of **2a** with NFSi and Selectfluor. Synthesis of compounds **7–9**. Reagents and conditions: (a) KHMDS (2.2 equiv.), NFSi (3.0 equiv.), THF, 4 Å MS, −78 °C, 1 h and 25 °C, 1 h, **2a** → **7** (33%), plus **9** (≤5%); (b) KHMDS (2.0 equiv.), Selectfluor (10.0 equiv.), THF–DMF (10 : 1), 4 Å MS, −78 °C, 1–2 h and 25 °C, 1 h, **2a** → **8** (24%), plus **9** (≤20%). KHMDS = potassium bis(trimethylsilyl)amide; NFSi = *N*-fluorobenzenesulfonimide; Selectfluor = 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate); DMF = *N*,*N*-dimethylformamide.



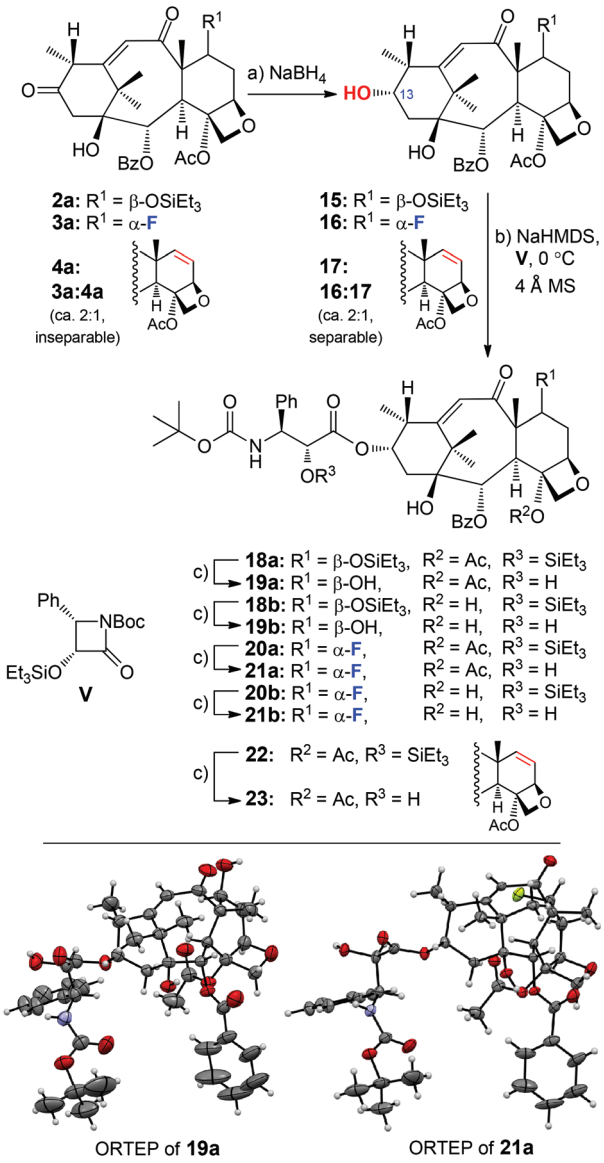
Scheme 4 Fluorination of **3a** with NFSi and Selectfluor. Synthesis of compounds **10–14**. Reagents and conditions: (a) KHMDS (2.2 equiv.), NFSi (3.0 equiv.), THF, 4 Å MS, −78 °C, 1 h and 25 °C, 1 h, **3a** → **10** (24%); (b) KHMDS (2.0 equiv.), Selectfluor (10.0 equiv.), THF–DMF (10 : 1), 4 Å MS, −78 °C, 1–2 h and 25 °C, 1 h, **3a** → **11** (≤5%), plus **12** (≤2%), plus **13** (17%); **13** → **14** (97%).

Scheme 1), and 7α,10α-difluoroenone **13** (17% yield). Further treatment of the latter with excess of Selectfluor under the same set of conditions led to trifluoro enone **14** (mp = 229–230 °C, CH_3Cl–hexanes–EtOAc 10 : 5 : 1) in 97% yield. The structure of **14** was unambiguously established by 2D NMR spectroscopic techniques and X-ray crystallographic analysis (see ORTEP drawing, Scheme 4).

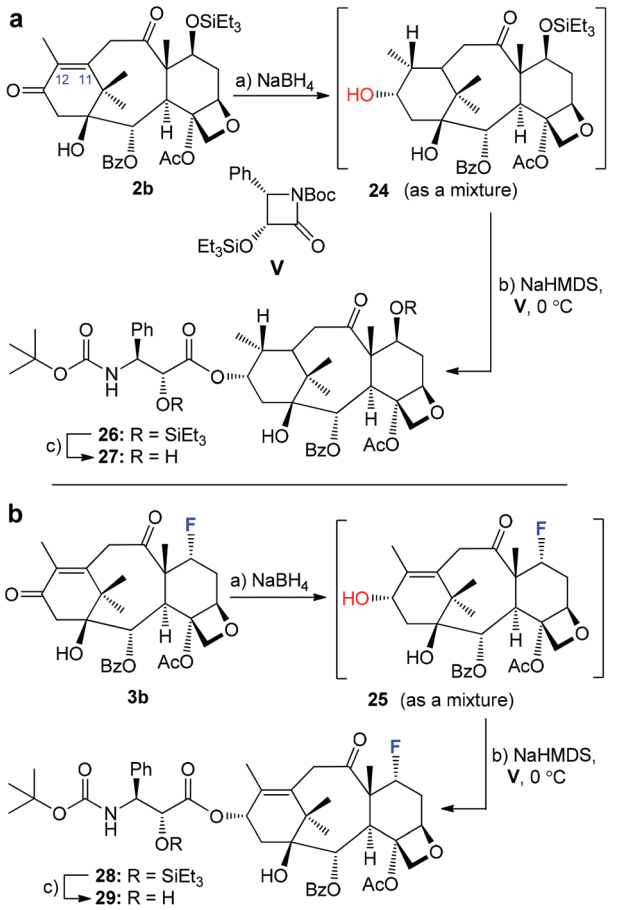
Having a variety of rearranged (**2a**, **2b**, **4a**) and fluorinated (**3a**, **3b**, **8**, **9**, **11–14**) taxoids in hand, we pursued their conversion to docetaxel analogs through reduction of their C-13 carbonyl moieties and attachment of the side chain.

Reduction of diketones 2a, 3a, and 4, and preparation of docetaxel analogs 19a, 19b, 21a, 21b and 23

As a prelude to the preparation of docetaxel analogs from the dicarbonyl compounds **2a**, **3a**, and **4a**, we subjected the latter to $NaBH_4$ reduction in the hope that the less hindered 13-carbonyl moiety would be preferentially and selectively reduced to the corresponding 13-hydroxy precursors. Indeed, exposure of **2a**, **3a** and **4a** (as a pure compound or a *ca.* 2 : 1 of **3a** : **4a** inseparable mixture) to $NaBH_4$ [2.5 equiv., THF–MeOH (1 : 2), 0 °C, procedure (a), Scheme 5] led to alcohols **15** (94%), **16** (71%), and **17** [30% yield after chromatographic separation from the concomitantly formed **16** (66% yield)], respectively, all possessing the desired 13α configuration as shown in Scheme 5. Each of these hydroxy compounds was acylated using procedure (b) (Scheme 5): NaHMDS (2.5 equiv.) and β-lactam side chain equivalent (3*R*,4*S*)-3-triethylsilanyloxy-4-phenyl-*N*-Boc-2-azetidinone (**V**) (5.0–10.0 equiv.) to afford 7,2′-bis-silylated-4-acetoxy products **18a** (53% yield), **20a** (53% yield), 2′-silylated-4-acetoxy-$\Delta^{7,8}$ product **22** (31% yield) or 7,2′-bis-silylated-4-deacetyl products **18b** (60% yield), and **20b** (52% yield), depending on the conditions employed (see ESI†). These compounds were then desilylated by exposure to conditions (c) (Scheme 5): aqueous HCl, THF–MeOH (1 : 2), 0 °C, leading to docetaxel analogs **19a** (83% yield), **19b** (89% yield), **21a** (96% yield), **21b** (73% yield), and **23** (94% yield). The absolute stereochemical configurations of **19a** (mp = 178–181 °C, CH_3CN) and **21a** (mp = 233–235 °C, CH_3CN–hexanes–EtOH 10 : 2 : 5) were confirmed by X-ray crystallographic analysis (see ORTEP representations of **19a** and **21a**, Scheme 5).



Scheme 5 Synthesis of compounds **15–17**, **18a–21a**, **18b–21b**, **22** and **23**. Reagents and conditions: (a) $NaBH_4$ (2.5 equiv.), THF–MeOH (1 : 2), 0 °C, 0.5–3 h, **2a** → **15** (94%); **3a** → **16** (71%); **3a** : **4a** (*ca.* 2 : 1 by ^1H NMR)→ **16** (66%), plus **17** (30%); (b) NaHMDS (2.5 equiv.), **V** (5.0–10.0 equiv.), THF, 4 Å MS, 0 °C, 40 min (or −78→ 0 °C, 40 min see ESI† for detailed procedure B1, B2, and B3), **15** → **18a** (53%); **15** → **18b** (60%); **16** → **20a** (53%); **16** → **20b** (52%); **17** → **22** (31%); (c) aq. HCl (1.0 M) (excess), THF–MeOH (1 : 2), 0 °C, 1–3 h, **18a** → **19a** (83%); **18b** → **19b** (89%); **20a** → **21a** (96%); **20b** → **21b** (73%); **22** → **23** (94%). NaHMDS = sodium bis(trimethylsilyl)amide; **V** = (3*R*,4*S*)-3-triethylsilanyloxy-4-phenyl-*N*-Boc-2-azetidinone.



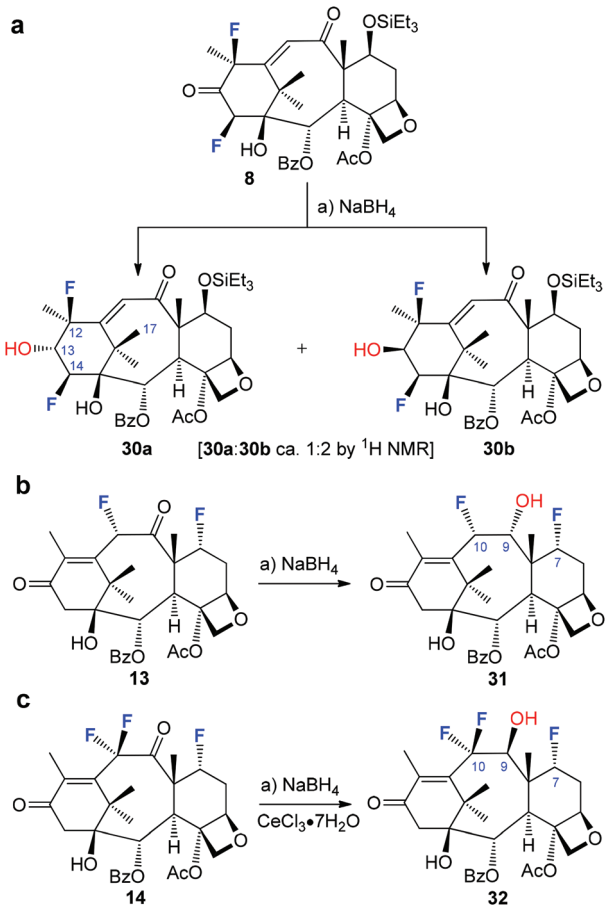
Scheme 6 Stereo- and regioselectivity of the reduction of ketones **2b** and **3b**. Synthesis of compounds **24–29**. Reagents and conditions: (a) $NaBH_4$ (2.5 equiv.→ large excess), THF–MeOH (1 : 2), 0 °C, 1–3 h; (b) NaHMDS (2.5 equiv.), **V** (5.0–10.0 equiv.), THF, 4 Å MS, 0 °C, 40 min, **24** (mixture)→ **26** (24%); **25** (mixture)→ **28** (30%); (c) aq. HCl (1.0 M), THF–MeOH (1 : 2), 0 °C, 1–3 h, **26** → **27** (58%); **28** → **29** (89%).

Reduction of diketones 2b and 3b and preparation of docetaxel analogs 27 and 29

In contrast to the smooth reduction of diketones **2a**, **3a**, and **4a** discussed above, the reaction of compounds **2b** and **3b** with $NaBH_4$ proved to be complicated and low yielding with regards to the desired 13α-hydroxy products. Thus, and as shown in Scheme 6a, treatment of **2b** with a large excess of $NaBH_4$ resulted, through a rather sluggish reaction, in the formation of a complex mixture, in which the over-reduced product **24** was detected as the major compound. The same complications arose in the case of fluorinated diketone **3b** (Scheme 6b) which led, upon treatment with excess of $NaBH_4$, to a complex mixture, in which the desired alcohol **25** (note the retention at the $\Delta^{11,12}$ olefinic bond as contrasted with **24**) was determined to be the predominant product. Unfortunately, neither product (*i.e.* **24** and **25**) could be isolated in pure form by flash chromatography (silica gel), forcing us to employ the crude mixtures in the side chain attachment step. Thus, treatment of the mixtures containing **24** or **25** (see Scheme 6) with NaHMDS and β-lactam **V** at 0 °C as described above [conditions (b)] furnished pure coupling products **26** (24% yield) or **28** (30% yield), respectively, in pure form after chromatographic separation. Desilylation of products **26** and **28** under conditions (c), (Scheme 6) [aq. HCl, THF–MeOH (1 : 2), 0 °C] led to docetaxel analogs **27** (58% yield) and **29** (89% yield), respectively.

Reduction of diketones 8, 13, and 14

Fluorinated diketones **8**, **13**, and **14** exhibited interesting reactivity towards $NaBH_4$ reduction as shown in Scheme 7. Thus,



Scheme 7 Stereo- and regioselectivity of the reduction of ketones **8**, **13**, and **14**. Synthesis of compounds **30a**, **30b**, **31**, and **32**. Reagents and conditions: (a) $NaBH_4$ (2.5 equiv.→ large excess), THF–MeOH (1 : 2), 0 °C, 0.5–2 h (or $NaBH_4$ (large excess), THF–MeOH (1 : 2), 0 °C, $CeCl_3{\cdot}7H_2O$ (cat.), see ESI†), **8** → **30a** (44%), plus **30b** (39%); **13** → **31** (58%); **14** → **32** (95%).

treatment of 12β,14β-difluorodiketone **9** with a large excess of $NaBH_4$ [THF–MeOH = 1 : 2, conditions (a)] resulted in the formation of a mixture of 13α- and 13β-hydroxyenones **30a** (44% yield) and **30b** (39% yield) (Scheme 7a). Apparently the replacement of the hydrogen atoms at the C-12 and C-14 positions with fluorines influences the stereoselectivity of this reduction (compare **2a** → **15**, Scheme 5). Even more interesting was the $NaBH_4$-mediated reduction of 7α,10α-difluorodiketone **13** (Scheme 7b) which led to 9α-hydroxyenone **31** (58% yield). In contrast, the reduction of the trifluoro diketone **14** (Scheme 7c) under the same conditions (a) proved sluggish and required Luche conditions ($NaBH_4$–$CeCl_3{\cdot}7H_2O$), leading to 9β-hydroxyenone **32** (95% yield). The observed reactivity enhancement of the C-9 carbonyl moiety over the C-13 carbonyl group within diketones **13** and **14** may be attributed to the electron-withdrawing properties of the fluorine substituents at C-7 and C-10 carbons adjacent to the former functional group. The opposite stereochemical outcome of this reduction in the case of **14**, as compared to that of diketone **13** (Scheme 7b), is presumably due to the steric effect of the 10β-fluorine residue in the latter, an outcome observed also in the reduction of **8**, which led partially to reversal of stereoselectivity (see **8** → **30a** + **30b**, Scheme 7a).

While these results were interesting on their own right, they precluded the attachment of the docetaxel side chain at the proper position of the products in order to obtain useful analogs for biological evaluation.

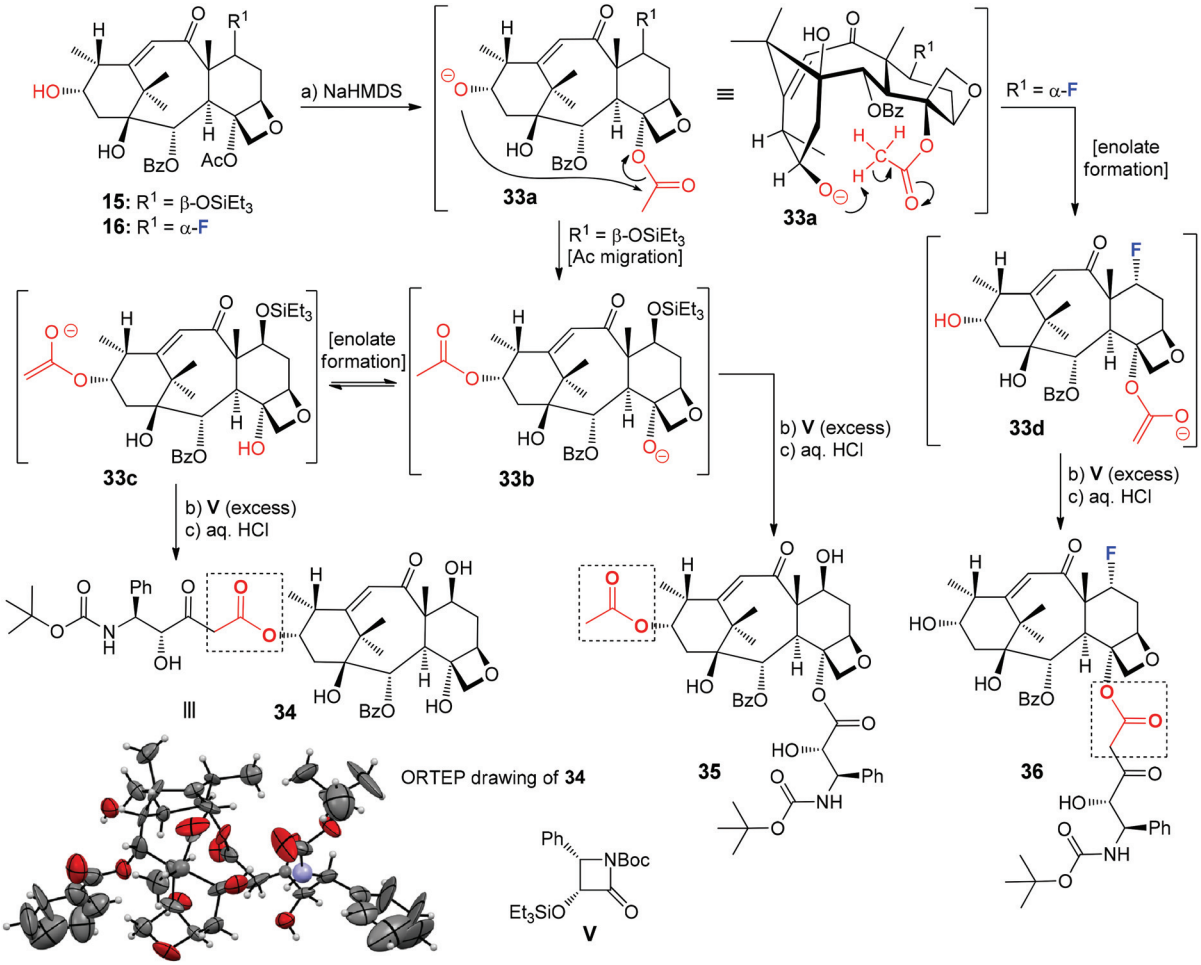
Preparation of unusual taxoids 34–36 from 15 and 16

While optimizing the conditions for the preparation of docetaxel analogs from hydroxy compounds **15** and **16**, we encountered a number of unusual side-products, including compounds **34**, **35**, and **36** (Scheme 8). These compounds were of interest not only from the biological point of view, but also for mechanistic considerations of their formation. Speculative pathways for their generation are outlined in Scheme 8. Thus, under the basic conditions (a) employed for the side chain attachment (*i.e.* NaHMDS), the C-13-alkoxide (**33a**) formed from **15** or **16** may undergo intramolecular rearrangement through C-4-acetyl migration as shown in the case of **15** (R^1 = β-OTES), or intramolecular enolate formation as shown in the case of **16** (R^1 = F) to afford acetate **33b** or enolate **33d**, respectively. The former may also rearrange to enolate **33c**. All three reactive species (*i.e.* **33c**, **33b**, and **33d**) may then react with β-lactam **V** to afford the corresponding esters, which upon acid-induced desilylation lead to compounds **34** (39% overall yield from **15**), **35** (12% overall yield from **15**), and **36** (16% overall yield from **16**), respectively as shown in Scheme 8. The absolute configuration of **34** (mp = 161–163 °C, hexanes–EtOAc 10 : 1) was confirmed by X-ray crystallographic analysis (see ORTEP representation, Scheme 8).

Biological evaluation of selected compounds

Selected compounds from those synthesized and described above, including sulfonates **7**, **10**, docetaxel analogs **19a**, **19b**, **21a**, **21b**, **23**, **27**, **29** and side-products **34–36** were submitted to the NCI 60 cell line screening program.[45] Initial one-dose screening (at 10 μM conc.) revealed that sulfonates **7**, **10**, and 7α-fluorinated compound **36** possessed weak cytotoxicity (Fig. 2). Specifically, sulfonate **7** demonstrated moderate results, however, not sufficient for the five-dose screening [98% growth inhibition (GI) for HOP-92 (non-small cell lung cancer) and 8% lethality for A498 (renal cancer)]. 7α-Fluorinated sulfonate **10** was less active [76% growth inhibition (GI) for MDA-MB-435 (melanoma), 66% growth inhibition for HT29 (colon cancer), and RPMI-8226 (leukemia)]. Compound **36** was the least active, suppressing the growth of CNS cancer cell line (SNB-75) by only 33%. While these activities did not warrant further investigation of the compounds mentioned above, those of the remaining did.

Thus, because of their significant potencies against numerous tumor cell lines, docetaxel analogs **19a**, **19b**, **21a**, **21b**, **23**, **27**, **29**, **34**, and **35** were advanced to the five-dose screening stage, revealing more details about their cytotoxicities as depicted in Fig. 2 [the most potent compounds are shown in boxes (*e.g.*, **23**, **27**, and **29**); the highest potencies are emphasized in red (less than 5 nM) and bold black (from 10 to 100



Scheme 8 Generation of compounds **34–36** and postulated mechanism for their formation from **15**, **16**, and **V**. Reagents and conditions: (a) NaHMDS (2.0–3.0 equiv.), –78 °C, 1 h, then (b) **V** (5.0–10.0 equiv.), THF, –78 °C, 0.5 h and 0 °C, 0.5 h, see ESI† for detailed procedures B2 and B3; then PTLC, followed by (c) aq. HCl (1.0 M) (excess), THF–MeOH (1 : 2), 0 °C, 1–3 h (yields reported over 3 steps), **15** → **34** (39%), plus **35** (12%); **16** → **36** (16%). PTLC = preparative thin-layer chromatography.

nM)]. Specifically, analogs **23**, **27**, and **29** demonstrated very low GI_{50} values (less than 5 nM) against most of the 60 cell lines tested: for **23** [for most of the leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines $GI_{50} \leq 5$ nM]; for **27** [for many of the leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, prostate cancer, and breast cancer cell lines $GI_{50} \leq 5$ nM]; for **29** [CNS cancer (SNB-75) $GI_{50} \leq 5$ nM; melanoma (MDA-MB-435) $GI_{50} \leq 5$ nM and (SK-MEL-5) GI_{50} = 7 nM; renal cancer (RXF 393) GI_{50} = 11 nM; breast cancer (HS 578T) GI_{50} = 7 nM]. Taxoids **19a**, **19b**, **21a**, and **21b** showed moderate cytotoxicity (GI_{50} values vary from 10 to 100 nM). In particular, for **19a** [for most of the leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines $GI_{50} \leq 10$ nM)]; for **19b** [leukemia (HL-60(TB)) GI_{50} = 184 nM; colon cancer (HT29) GI_{50} = 188 nM; CNS cancer (SNB-75) GI_{50} = 25 nM; melanoma (MDA-MB-435) GI_{50} = 76 nM; breast cancer (MCF7) GI_{50} = 188 nM and (HS 578T) GI_{50} = 137 nM]; for **21a** [CNS cancer (SNB-75) $GI_{50} \leq 10$ nM; melanoma (MDA-MB-435) GI_{50} = 13 nM; renal cancer (RXF 393) GI_{50} = 24 nM; breast cancer (HS 578T) GI_{50} = 21 nM and (MDA-MB-468)] GI_{50} = 18 nM]; and for **21b** [non-small cell lung cancer (NCI-H460) GI_{50} = 210 nM; colon cancer (HT29) GI_{50} = 259 nM; CNS cancer (SNB-75) GI_{50} = 87 nM; melanoma (MDA-MB-435) GI_{50} = 144 nM; breast cancer (MCF7) GI_{50} = 295 nM]. Analogs **34** and **35** exhibited low cytotoxicities ($GI_{50} \geq 500$ nM for most of the 60 cell lines) as shown in Fig. 2. For further details of the cytotoxicity data see http://dtp.nci.nih.gov/.

Conclusion

An exploration of the reactivity of the readily available taxoid 10-deacetylbaccatin III towards various fluorinating reagents led to a number of novel baccatin III derivatives, some of which were successfully converted to docetaxel analogs through reduction and side chain attachment. Biological evaluation of these analogs at NCI employing their 60 cell line

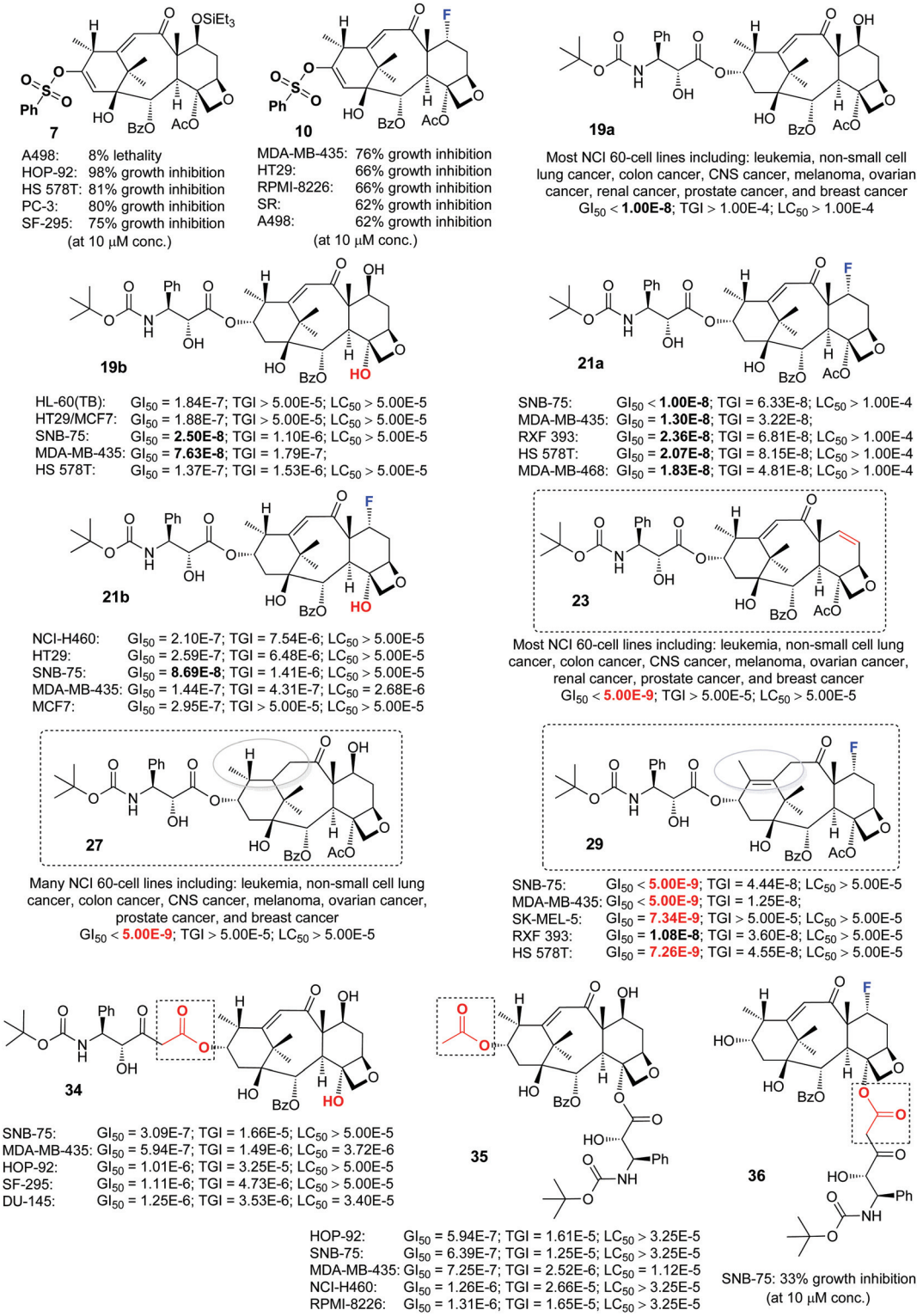


Fig. 2 Cytotoxicities of chosen synthesized taxoids against selected cancer cell lines (NCI). For experimental details and definitions, please use the following weblink: (http://dtp.nci.nih.gov/branches/btb/ivclsp.html). GI_{50} = growth inhibition of 50%, *i.e.* drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation; TGI = drug concentration resulting in total growth inhibition; LC_{50} = concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning; SRB = sulforhodamine B. Units: molar. Color code: bold red (GI_{50} < 5 nM) and bold black (GI_{50} = 10–100 nM) numbers indicate high potencies. Average GI_{50} for paclitaxel for most cell lines varies from 10–35 nM. For further details on all compounds, see ESI† and http://dtp.nci.nih.gov/ (see Table S1† for compound numbering).

screening assays led to the identification of a number of highly potent compounds against several cancerous cell lines (*i.e.* **23**, **27**, and **29**; $GI_{50} \leq 5$ nM). These results indicate that oxygenation at C-7 and C-10 of the taxoid family is not necessary for biological activity. They also suggest that some of the reported compounds warrant further investigation aiming at their development as personalized medicines for the treatment of certain cancers.

Abbreviations

DAST	(Diethylamino)sulfurtrifluoride
TES	Triethylsilyl
MS	Molecular sieves
THF	Tetrahydrofuran
4-DMAP	4-(Dimethylamino)pyridine
KHMDS	Potassium bis(trimethylsilyl)amide
NFSi	*N*-Fluorobenzenesulfonimide
DMF	*N*,*N*-Dimethylformamide
NaHMDS	Sodium bis(trimethylsilyl)amide
PTLC	Preparative thin-layer chromatography

Acknowledgements

We thank Drs D. H. Huang and L. Pasternack for NMR spectroscopic, Dr G. Siuzdak for mass spectrometric, and Dr R. K. Chadha for X-ray crystallographic assistance. Financial support for this work was provided by the Skaggs Institute for Research and the National Institutes of Health, USA (Grant AI055475). We are grateful to Merck for a postdoctoral fellowship (to R. A. V.). We also thank Armong Chen, Fujian South Pharmaceutical Co., Ltd. (Shanghai ParlingPharmTech Co., Ltd) for generous gifts of 10-deacetylbaccatin III (**III**) and lactam **V**, the National Cancer Institute (NCI) for performing the biological experiments and obtaining the data reported in this publication, and Drs Doug Smallwood and Mohammed Nayel of the NCI for assistance and helpful discussions.

References

1 F. Bray, A. Jemal, N. Grey, J. Ferlay and D. Forman, *Lancet Oncol.*, 2012, **13**, 790–801.
2 K. C. Nicolaou, W.-M. Dai and R. K. Guy, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 15–44.
3 U.S. Department of Health and Human Services, *Medical Subject Headings, Tree Structures, 2003*, National Library of Medicine, 2002, pp. 193–220.
4 V. T. DeVita Jr, S. Hellman and S. A. Rosenberg, in *Cancer: Principles and Practice of Oncology*, Lippincott Williams & Wilkins, 7th edn, 2005, vol. 1, pp. 1–1488, vol. 2, pp. 1489–2898.
5 *Cancer Facts & Figures 2012*, American Cancer Society, Atlanta, 2012, pp. 1–64.
6 http://disease-ontology.org/
7 K. C. Nicolaou and T. Montagnon, in *Molecules that Changed the World*, Wiley-VCH, Weinheim, 2008, pp. 1–366.
8 K. C. Nicolaou, J. S. Chen and S. M. Dalby, *Bioorg. Med. Chem.*, 2009, **17**, 2290–2303.
9 M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon and A. T. McPhail, *J. Am. Chem. Soc.*, 1971, **93**, 2325–2327.
10 Y.-F. Wang, Q.-W. Shi, M. Dong, H. Kiyota, Y.-C. Gu and B. Cong, *Chem. Rev.*, 2011, **111**, 7652–7709.
11 P. B. Schiff, J. Fant and S. B. Horwitz, *Nature*, 1979, **277**, 665–667.
12 http://www.cancer.gov/cancertopics/druginfo/paclitaxel
13 http://www.cancer.gov/cancertopics/druginfo/docetaxel
14 K. A. Lyseng-Williamson and C. Fenton, *Drugs*, 2005, **65**, 2513–2531.
15 P. A. Arroyo, R. U. Pérez, B. C. Díez, M. A. F. Feijóo, J. H. Magdalena and M. A. C. Hernández, *Cancer Treat. Rev.*, 2011, **37**, 105–110.
16 G. Samaranayake, K. A. Neidigh and D. G. I. Kingston, *J. Nat. Prod.*, 1993, **56**, 884–898.
17 I. Ojima, O. Duclos, M. Zucco, M.-C. Bissery, C. Combeau, P. Vrignaud, J. F. Riou and F. Lavelle, *J. Med. Chem.*, 1994, **37**, 2602–2608.
18 I. Ojima, P.-Y. Bounaud, C. Takeuchi, P. Pera and R. J. Bernacki, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 189–194.
19 J. Dubois, S. Thoret, F. Guéritte and D. Guénard, *Tetrahedron Lett.*, 2000, **41**, 3331–3334.
20 Q. Cheng, T. Oritani and T. Horiguchi, *Tetrahedron*, 2000, **56**, 1667–1679.
21 S. Iimura, K. Uoto, S. Ohsuki, J. Chiba, T. Yoshino, M. Iwahana, T. Jimbo, H. Terasawa and T. Soga, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 407–410.
22 J. Kant, W. S. Schwartz, C. Fairchild, Q. Gao, S. Huang, B. H. Long, J. F. Kadow, D. R. Langley, V. Farina and D. Vyas, *Tetrahedron Lett.*, 1996, **37**, 6495–6498.
23 I. Ojima, S. D. Kuduk, P. Pera, J. M. Veith and R. J. Bernacki, *J. Med. Chem.*, 1997, **40**, 279–285.
24 O. Querolle, J. Dubois, S. Thoret, F. Roussi, S. Montiel-Smith, F. Guéritte and D. Guénard, *J. Med. Chem.*, 2003, **46**, 3623–3630.
25 I. Ojima, C. L. Fumero-Oderda, S. D. Kuduk, Z. Ma, F. Kirikae and T. Kirikae, *Bioorg. Med. Chem.*, 2003, **11**, 2867–2888.
26 O. Querolle, J. Dubois, S. Thoret, F. Roussi, F. Guéritte and D. Guénard, *J. Med. Chem.*, 2004, **47**, 5937–5944.
27 B. Ke, Y. Qin, F. Zhao and Y. Qu, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4783–4785.
28 H.-F. Lu, C. Xie, J. Chang, G.-Q. Lin and X. Sun, *Eur. J. Med. Chem.*, 2011, **46**, 1743–1748.
29 G. Samaranayake, N. F. Magri, C. Jitrangsri and D. G. I. Kingston, *J. Org. Chem.*, 1991, **56**, 5114–5119.
30 A. Wahl, F. Guéritte-Voegelein, D. Guénard, M.-T. Le Goff and P. Potier, *Tetrahedron*, 1992, **48**, 6965–6974.
31 S.-H. Chen, S. Huang, J. Wei and V. Farina, *Tetrahedron*, 1993, **49**, 2805–2828.
32 J.-P. Pulicani, B. Hervé, J.-D. Bourzat and A. Commerçon, *Tetrahedron Lett.*, 1994, **35**, 9709–9712.

33 S.-H. Chen, V. Farina, S. Huang, Q. Gao, J. Golik and T. W. Doyle, *Tetrahedron*, 1994, **50**, 8633–8650.
34 S.-H. Chen, S. Huang, Q. Gao, J. Golik and V. Farina, *J. Org. Chem.*, 1994, **59**, 1475–1484.
35 C. Yu and Z. Liu, *Tetrahedron Lett.*, 1997, **38**, 4133–4136.
36 R. A. Johnson, P. J. Dobrowolski, E. G. Nidy, I. Gebhard, S. J. Qualls, N. A. Wicnienski, R. C. Kelly, T. W. Pitts, N. M. Lopes, T. F. DeKoning, S. J. Mattern, S. D. Weed, J. P. McGovren and C. G. Chidester, *J. Med. Chem.*, 1997, **40**, 2810–2812.
37 Y. Zhao, H.-B. Zhang, J.-K. Liu, J. Su, Y. Li, Z.-J. Yao and Q.-S. Zhao, *Tetrahedron Lett.*, 2011, **52**, 139–142.
38 F. Gao, Z.-K. Yang, Q.-H. Chen, X.-G. Chen and F.-P. Wang, *Org. Biomol. Chem.*, 2012, **10**, 361–366.
39 J. Fried and E. F. Sabo, *J. Am. Chem. Soc.*, 1954, **76**, 1455–1456.
40 P. A. Bartlett and A. Otake, *J. Org. Chem.*, 1995, **60**, 3107–3111.
41 C. Isanbor and D. O'Hagan, *J. Fluorine Chem.*, 2006, **127**, 303–319.
42 K. L. Kirk, *J. Fluorine Chem.*, 2006, **127**, 1013–1029.
43 D. O'Hagan, *J. Fluorine Chem.*, 2010, **131**, 1071–1081.
44 L. E. Zimmer, C. Sparr and R. Gilmour, *Angew. Chem., Int. Ed.*, 2011, **50**, 11860–11871.
45 http://dtp.nci.nih.gov/
46 L. A. Paquette, D. Crich, P. L. Fuchs and G. Molander, in *Encyclopedia of Reagents for Organic Synthesis*, John Wiley & Sons, 2nd edn, 2009, pp. 1–12094 (14 volumes).
47 Albeit this particular conversion was previously unknown, Kant *et al*. from Bristol-Myers Squibb Research Institute observed similar undesired side-product while preparing C-10 Taxol® analogs: J. Kant, W. S. O'Keeffe, S.-H. Chen, V. Farina, C. Fairchild, K. Johnston, J. F. Kadow, B. H. Long and D. Vyas, *Tetrahedron Lett.*, 1994, **35**, 5543–5546.
48 J.-N. Denis and A. E. Greene, *J. Am. Chem. Soc.*, 1988, **110**, 5917–5919.
49 Intermediate **5d**, under warmer temperature and prolonged treatment, is prone to rearrangement, producing taxyunansin-related[37] motif **6** through A-ring contraction, as previously reported by Kingston,[29] Liu,[35] and Chen.[50,51] In the presence of moisture **5d** hydrolyzes, forming a mixture of two diastereomeric sulfinamides (**7** or **7′**, which were observed exclusively in the reaction between 13-acetylbaccatin III and DAST):[51]S.-H. Chen, S. Huang, J. Wei and V. Farina, *J. Org. Chem.*, 1993, **58**, 4520–4521.
50 S.-H. Chen, S. Huang and V. Farina, *Tetrahedron Lett.*, 1994, **35**, 41–44.
51 G. P. Roth, D. R. Marshall and S.-H. Chen, *Tetrahedron Lett.*, 1995, **36**, 1609–1612.
52 No cyclopropane-containing compound was isolated in the reaction, see: R. A. Johnson, E. G. Nidy, P. J. Dobrowolski, I. Gebhard, S. J. Qualls, N. A. Wicnienski and R. C. Kelly, *Tetrahedron Lett.*, 1994, **35**, 7893–7896; see also ref. 49–51.
53 M. R. C. Gerstenberger and A. Haas, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 647–667.
54 G. S. Lal, G. P. Pez and R. G. Syvret, *Chem. Rev.*, 1996, **96**, 1737–1755.
55 J.-A. Ma and D. Cahard, *Chem. Rev.*, 2004, **104**, 6119–6146.
56 J.-A. Ma and D. Cahard, *J. Fluorine Chem.*, 2007, **128**, 975–996.
57 P. T. Nyffeler, S. G. Durón, M. D. Burkart, S. P. Vincent and C.-H. Wong, *Angew. Chem., Int. Ed.*, 2005, **44**, 192–212.
58 Chen's group reported the formation of α-fluorinated enone (at C-12, related to **8**) together with similar dienone by using Yarovenko's reagent ($ClFHCCF_2NEt_2$): S.-H. Chen, C. Fairchild, S. W. Mamber and V. Farina, *J. Org. Chem.*, 1993, **58**, 2927–2928.