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Novel 3′,6′-Anhydro and N12,N13-Bridged Glycosylated Fluoroindolo[2,3-*a*]carbazoles as Topoisomerase I Inhibitors. Fluorine as a Leaving Group from sp^3 Carbon

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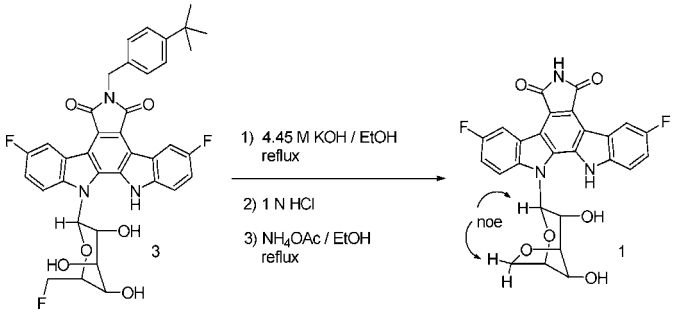
ABSTRACT



Both 6′- and 4′-fluoro-glycosylated indolo[2,3-*a*]carbazoles are substrates for base-induced loss of fluorine as a leaving group from sp^3 carbon. In the case of α-N-glycosylated substrate 3, loss of fluorine from the 6′-position leads to 3,6-anhydroglucose analogue 1. A novel N12,N13-bridged sugar analogue 2 results from loss of 4′-fluorine from *β*-N-glycosylated analogue 4. Both analogues 1 and 2 display topo I inhibitory potencies similar to camptothecin.

The glycosylated indolo[2,3-*a*]carbazole represents a pharmacophore that has been identified to have potent anticancer activity.[1] This class manifests its cytotoxicity through a diverse array of anticancer targets. For example, protein kinase A and protein kinase C (PKC) are targeted by staurosporine, and 7-hydroxy-staurosporine (UCN-01) is presently undergoing clinical trials for cancer treatment.[2] We recently described a series of fluoro-glycosylated fluoroindolocarbazoles from which BMS-250749 emerged as the lead clinical candidate as a result of its broad spectrum antitumor activity against preclinical xenograft models.[3]

This series of fluoroindolocarbazoles derives its mechanism of action from inhibition of the nuclear enzyme topoisomerase I (topo I).[4] Topo I is a ubiquitous enzyme that interconverts supercoiled and relaxed DNA in the

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(1) (a) Long, B. H.; Balasubramanian, B. N. *Exp. Opin. Ther. Patents* **2000**, *10* (5), 635−666. (b) Prudhomme, M. *Curr. Pharm. Des.* **1997**, *3*, 265.

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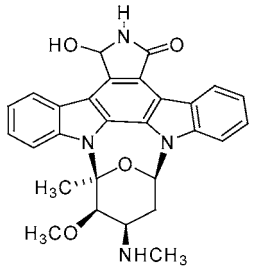
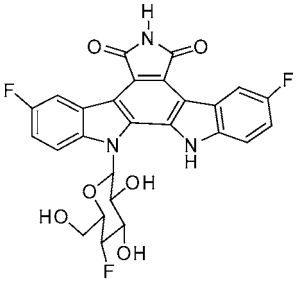
maintenance of DNA topology during the processes of replication and transcription. Topo I catalyzes the transient break of one strand of duplex DNA and allows the unbroken complementary strand to pass through the enzyme-linked strand, resulting in DNA relaxation by one positive turn. Inhibitors of topoisomerase I such as the fluoroindolocarbazoles and camptothecin (CPT) stabilize this enzyme-linked break, resulting in a "cleavable terniary complex", thereby preventing the normal religation process. These "natural transient DNA breaks" are thereby made permanent, with the result that the DNA cleavage induces cellular cytotoxicity and apoptosis.[5] Topo I is a clinically proven target for antitumor activity, as demonstrated by the marketed CPT analogues, CPT-11 and topotecan.[6]



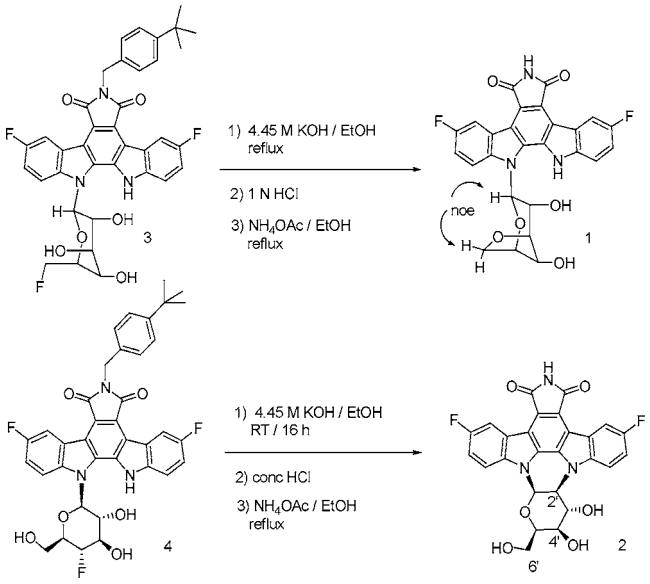
UCN-01



BMS-250749

Introduction of fluorine into the β-N-12 glucose ring of the fluoroindolocarbazole imparts distal antitumor activity.[3b] In the context of the synthesis of these fluoroglucose analogues, we discovered some rather surprising chemistry, whereby both 6′- and 4′-fluorine function as a leaving group from sp^3 carbon. While fluorine is considered a poor leaving group from sp^3 carbon (relative rate for F^- of 0.0001 vs 1 for Br^-),[7] there are rare examples such as epoxides from vicinal fluorohydrins in steroid chemistry[8] and quinone methide formation via fluoride ion ejection from (2-trifluoromethyl)phenylacetonitrile.[9] We now describe the synthesis of the 3,6-anhydroglucose analogue **1**, and the staurosporine-like N12,N13-bridged analogue **2**, via base-induced loss of fluoride from the corresponding 6′- and 4′-fluoroglucose intermediates, **3** and **4**, respectively (Scheme 1).

Scheme 1. Fluorine as a Leaving Group from 6′- and 4′-Fluoroglucosyl-fluoroindolo[2,3-*a*]carbazole Analogues



We previously reported the deprotection of the *N*-6-imide (4-*tert*-butyl)benzyl group via a three step, one-pot sequence, involving (1) aqueous ethanolic potassium hydroxide-induced imide hydrolysis, (2) acidification with concentrated hydrochloric acid to give the anhydride, and (3) N-6-unsubstituted imide formation using ammonium acetate in ethanol.[3] This three step, one-pot sequence for imide deprotection was routinely utilized for a series of fluoroindolocarbazole analogues (vide supra).[3] During imide deprotection of the 6′-fluoro α-anomer **3**,[10] the desired 6′-fluoro unsubstituted imide was not obtained. Instead 6′-fluorine was lost via intramolecular nucleophilic attack by the 3′-hydroxyl, giving 3′,6′-anhydroglucose analogue **1**[11] in 46% yield. Cyclization occurs during refluxing ethanolic potassium hydroxide and proceeds from the more stable chair conformation of anion **3a**, whereby all substituents are axial, except for the equatorial orientation of the fluoroindolocarbazole "core" at C1′. Molecular mechanics calculations using CHARMM[12] show that the difference in energy between the two chair conformations **3a** and **3b** is 5.11 kcal/mol as shown in Scheme 2. Chair conformer **3b** is higher in energy than **3a**

(3) (a) Balasubramanian, B. N.; St. Laurent, D. N.; Saulnier, M. G.; Long, B. H.; Bachand, C.; Beaulieu, F.; Clarke, W.; Deshpande, M.; Eummer, J. T.; Fairchild, C.; Frennesson, D. B.; Kramer, R.; Lee, F. Y.; Mahler, M.; Martel, A.; Naidu, B. N.; Rose, W. C.; Russell, J.; Ruediger, E.; Solomon, C.; Stoffan, K. M.; Wong, H.; Zimmermann, K.; Vyas, D. M. *J. Med Chem.* **2004**, *47*, 1609. (b) Saulnier, M. G.; Balasubramanian, B. N.; Long, B. H.; Frennesson, D. F.; Ruediger, E.; Zimmermann, K.; Eummer, J. T.; St. Laurent, D. N.; Stoffan, K. M.; Naidu, B. N.; Mahler, M.; Beaulieu, F.; Bachand, C.; Lee, F. Y.; Fairchild, C.; Stadnick, L. K.; Rose, W. C.; Solomon, C.; Wong, H.; Martel, A.; Kramer, R.; Langley, D. R.; Vyas, D. M. *J. Med. Chem.* **2005**, ASAP (DOI: 10.1021/jm049090z).

(4) Topoisomerase I is also targeted by the indolo[2,3-*a*]carbazoles NB-506 and ED-749, both of which are presently undergoing clinical evaluation. See: Yoshinari, T.; Ohkubo, M.; Fukasawa, K.; Egashira, S.; Hara, Y.; Matsumoto, M.; Nakai, K.; Arakawa, H.; Morishima, H.; Nishimura, S. *Cancer Res.* **1999**, *59*, 4271.

(5) Facompre, M.; Goossens, J.-F.; Bailly, C. *Biochem. Pharmacol.* **2001**, *61* (3), 299.

(6) In one of the quickest reviews ever seen, the FDA approved the use of CPT-11 in June 1996 as a second-line treatment for resistant colorectal cancer.

(7) Streitwieser, A. *Chem. Rev.* **1956**, *56*, 571.

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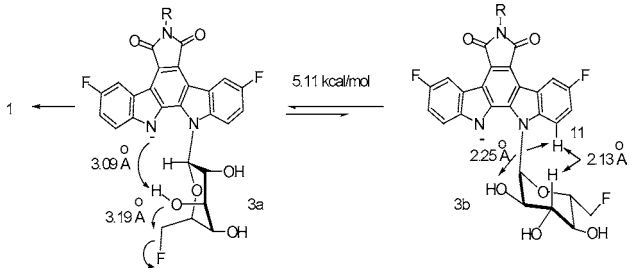
(10) 6′-Fluoro-α-anomer **3** was obtained in 6% yield from Misunobu reaction of the 3,9-difluoroindolocarbazole "core" with 6′-fluoro-2′,3′,4′-tri-*O*-benzyl-D-glucopyranose. The major β-isomer was obtained in 33% yield. See ref 3b. The 6′-fluoro-β-anomer of **3** is stable (6′-fluorine is not lost) under the reaction conditions (Scheme 1). This result is consistent with the more stable (3.35 kcal/mol) all-equatorial chair and the inability of the all-axial chair conformer to place the 3′-hydroxyl near the indole anion. See ref 12.

(11) Compounds **1**, **2**, and **6** were purified by flash chromatography on silica gel. These analogues gave HRMS consistent with their assigned structures.

(12) The ground-state conformer of **3a** (protonated) is calculated to be 7.85 kcal/mol lower in energy than the alternative **3b** (protonated) conformer. The global minimum for each compound was identified using a systematic grid search followed by CHARMM minimization (Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187) using the QUANTA force field (Momany, F. A.; Rone, R. *J. Comput. Chem.* **1992**, *13*, 888).

Scheme 2. Energy Difference in Chair Conformations of the Anion of **3** in the Proposed Mechanism for the Formation of **1** via 6′-Fluorine as a Leaving Group



due to steric interactions from the axially oriented indolocarbazole C11 ring hydrogen and the sugar C3′ hydrogen and O2′ hydroxyl. The **3a** conformer is further stabilized by a weak hydrogen bond between the indole anion and the axial 3′-hydroxyl. It is this interaction that may trigger the cyclization reaction to give **1**. The structure of **1** is secured by MS and COSY NMR data, including the observation of an NOE enhancement between the C1′ proton (6.75 ppm) and the C6′ proton (4.41 ppm).[13]

Intermediate **4** (Scheme 1) is penultimate to our clinical candidate, BMS-250749.[3b] Under imide deprotection conditions (vide supra), **4** is not converted to BMS-250749. The C4′-equatorial glucose fluorine does not survive the reaction conditions, even when the ethanolic potassium hydroxide reaction is conducted at room temperature. Instead, the N12,-N13-bridged sugar analogue **2** is isolated in 62% yield.[11] The structure of **2** is established by COSY NMR and NOE experiments.[14] The NOEs for **2** are shown in Figure 1. Single-

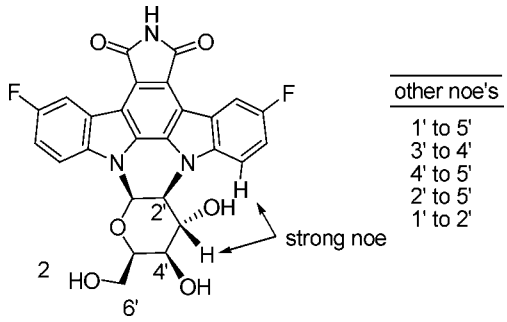


Figure 1. NOE enhancements for analogue **2**.

crystal X-ray analysis confirms the NMR assignment, and the ORTEP drawing of **2** is presented in Figure 2, showing

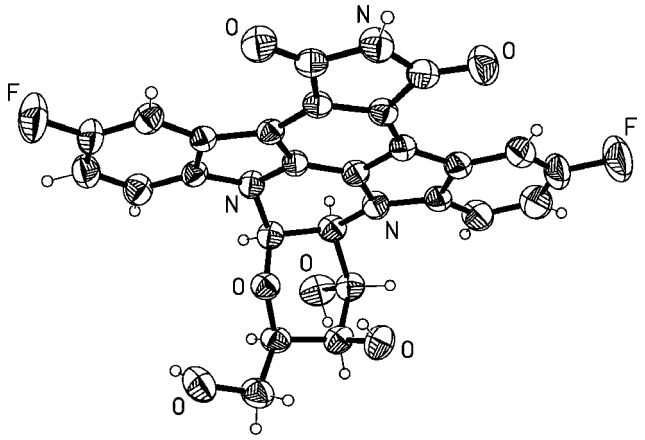


Figure 2. ORTEP drawing of **2** with ellipsoids drawn at the 50% probability level and H atoms arbitrarily scaled.

one of two crystallographically independent molecules in the asymmetric unit.[15]

The mechanism for the formation of the N12,N13-bridged analogue **2** from 4′-fluoroglucose **4** presumes that the 4′-fluorine was displaced with inversion of configuration by the adjacent 3′-hydroxyl to give a *β*-3′,4-epoxide. Payne rearrangement to the α-2′,3′-epoxide precedes nucleophilic attack by N13 on C2′ with inversion of configuration, yielding the observed product **2**. Such nucleophilic attack by N13 on C2′ with inversion of configuration is further precedented and exemplified by conversion of the 3′,4′,6′-tri-*O*-benzyl-D-glucopyranosyl-indolocabazole 2′-mesylate **5** to the N12,N13-bridged analogue **6** as shown below in Scheme 3.[11,16,17] This type of C-2′ activation followed by nucleophilic displacement by N13 was also noted by Prudhomme[18] using the 2′-tosylate of rebeccamycin, resulting in rebeccamycin analogues related to **6**. Several such analogues are described by Prudhomme as relatively weak topo I

(13) Stereochemistry of the sugar in **1** was determined on the basis of the proton−proton coupling constants and NOE experiments. Data for **1**: 500 MHz COSY ^{1}H NMR (DMSO-d_6) δ 8.86 (d, 1H, J = 9.6, 2.7 Hz), 8.75 (dd, 1H, J = 9.7, 2.6 Hz), 7.76 (dd, 1H, J = 9.1, 4.4 Hz), 7.72 (dd, 1H, J = 8.9, 4.5 Hz), 7.48 (ddd, 1H, J = 9.1, 9.0, 2.7 Hz), 7.44 (ddd, 1H, J = 9.0, 8.9, 2.8 Hz), 7.00 (d, 1H, J = 7.7 Hz, 1′H), 4.58−4.55 (m, 2H, 3′,6′H), 4.45 (d, 1H, J = 7.7 Hz, 2′H), 4.16 (d, 1H, J = 5.2 Hz, 6″H), 4.10 (d, 1H, J = 9.9 Hz, 5′H), 4.01−3.99 (d, 1H, 4′H); HRMS (neg ESI, M − H$^-$) calcd for $C_{26}H_{17}F2N_3O_6$ 504.1164, found 506.1155.

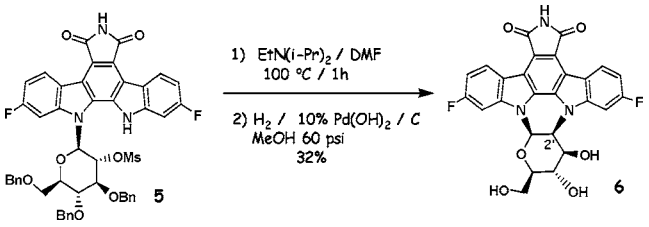
(14) Data for **2**: 500 MHz COSY ^{1}H NMR (DMSO-d_6) δ 11.04 (brs, 1H), 8.32 (dd, 1H, J = 9.1, 2.8 Hz), 8.22 (dd, 1H, J = 9.1, 2.6 Hz), 8.06 (dd, 1H, J = 9.0, 4.4 Hz), 7.93 (dd, 1H, J = 9.3, 4.3 Hz), 7.45−7.35 (m, 2H), 6.72 (brs, 1H, 1′H), 6.13 (brs, 1H, 3′OH), 5.38 (brs, 1H, 3′H), 4.77 (brs, 1H, 2′H), 4.73 (d, 1H, 4′OH, J = 5.5 Hz), 4.73 (t, 1H, 6′OH, J = 5.4 Hz), 4.28 (dd, 1H, 5′H, J = 6.2, 6.1 Hz), 3.71 (brs, 1H, 4′H), 3.38−3.18 (m, 2H, 6′H, 6″H); HRMS (neg ESI, M − H$^-$) calcd for $C_{26}H_{17}F2N_3O_6$ 504.1164, found 506.1170.

(15) The structure of **2** was determined by single-crystal X-ray analysis. The absolute configuration of the molecule was assigned on the basis of the refinement of the Flack parameter. Yellow rodlike crystals were grown from a solution of MeOH/EtOH/CH_2Cl_2 and were found to be a mono MeOH solvate of the compound. Two crystallographically independent molecules exist in each asymmetric unit and have the same conformation. The crystal structure exhibits a three-dimensional framework constructed by intermolecular hydrogen bonds involving both the parent and solvent molecules. Full crystallographic data have been deposited at the Cambridge Crystallographic Data Center (CCDC reference number 259792). Copies of the data can be obtained free of charge via the Internet at http://www.ccdc.cam.ac.uk.

(16) Mesylate **5** was prepared in 73% yield from the corresponding 2′-hydroxyl intermediate (described in ref 3a) using MsCl in pyridine at 0 °C.

(17) Data for **6**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.09 (s, 1H), 8.81 (d, J = 10.8 Hz, 1H), 8.73 (dd, J = 8.3, 6.4 Hz, 1H), 8.61 (dd, J = 8.4, 5.6 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.34−7.27 (m, 2H), 6.84 (d, J = 4.6 Hz, 1H), 6.80 (s, 1H), 5.64 (d, J = 5.1 Hz, 1H), 5.15 (s, 1H), 4.40−4.33 (m, 2H), 3.70−3.68 (m, 1H), 3.59−3.58 (m, 1H), 3.52−3.48 (m, 1H), 3.37−3.25 (m, 1H); MS (*m*/*e*) 504 (M − H)$^-$.

Scheme 3. N13−C2′ Bond Formation by Inversion of Configuration of the C2′-Mesylate in **5**



inhibitors, but many were efficient in their antiproliferative action against four tumor cell lines in vitro.[18b]

Our assay for determining the extent of topo I-mediated DNA cleavage for the analogues described herein employs covalently closed, supercoiled phage PM2 DNA as the substrate and was previously described.[3] The topo I selectivity of these analogues is determined as previously reported[3] using P388 cells that express high levels of topo I (and are therefore sensitive to topo I targeting agents such as CPT) and P388/CPT45 cells that do not express topo I and are thus resistant to topo I active agents such as CPT. A ratio (R/S) of the IC_{50} values for P388/CPT45 versus P388 cells for an analogue is an indication of topo I selectivity expressed by the analogue. The topo I activity (for DNA breaks), P388 cell cytotoxic potency, and R/S ratio of analogues **1**, **2**, and **6** are reported in Table 1.

Inspection of Table 1 shows that 3′,6′-anhydroglucose analogue **1** is about 3-fold less topo I active than CPT in its ability to induce single-strand breaks in the DNA substrate and is also about 2-fold less cytotoxic than CPT against P388 cells. The 3′,6′-anhydroglucose motif results in about 12-fold lower topo I potency vis-à-vis the parent D-glucose analogue in this fluoroindolocarbazole series (data not shown).[3] The N12,N13-bridged sugar analogue **2** is only about 2-fold less topo I potent than CPT yet is more cytotoxic against P388 cells than CPT. Analogue **2** also shows good topo I-selective cytotoxicity, as indicated by its resistance ratio (R/S), since it is not very cytotoxic against P388/CPT45 cells that are devoid of topo I expression and are thus resistant to topo I active agents such as CPT.

Table 1. Topo I Potency and Cytotoxicity of Analogues **1**, **2**, and **6**

analogue	topo I EC_{50}	potency ratio[a]	P388 IC_{50}	potency ratio[b]	R/S[c]
1	0.360	2.81	0.0388	1.83	36.6
2	0.110	1.77	0.0142	0.67	83.9
6	>100	>101	1.151	49	5.6
BMS-250749	0.019	0.11	0.0019	0.062	380

[a] Topoisomerase I potency relative to Camptothecin (analogue/CPT) determined in vitro. Values <1 are for analogues of greater potency than CPT. EC_{50} values are given in μM. [b] Cytotoxic potency relative to Camptothecin (analogue/CPT) determined for P388 cells. Values <1 are for analogues more potent than CPT. IC_{50} values are given in μM. [c] R/S = resistance ratio describing the topo I-selective cytotoxicity as a ratio of P388(R)/P388(S). Analogues with a low R/S ratio show low topo I-selective killing.

For the N12,N13-bridged analogues **2** and **6**, topo I potency and cytotoxicity are strongly dependent upon the stereochemistry of the C3′- and C4′-hydroxyls: the 3′,4′-"S,R" stereochemistry, as in **2**, results in good topo I potency and cytotoxicity, whereas the 3′,4′-"R, S" stereochemistry, as in **6**, results in complete loss of topo I potency and about 100-fold loss of cytotoxicity.[19] Such subtle changes in the stereochemical arrangement of the substituents decorating the sugar motif of these N12,N13-bridged glycosylated fluoroindolocarbazoles evidently can dictate a vast difference in the ability of these molecules to inhibit topo I. Additional examples of novel glycosylated analogues in this series of fluoroindolocarbazoles will be the subject of our future reports.

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(19) We have previously reported the effect of the "core" fluorine substitution pattern on topo I potency, selectivity, and cytotoxicity, with the result that 3,9-difluoro substitution (as in **2**) improves topo I potency by about about 9-fold over 2,10-difluoro substitution (as in **6**). The effect of core fluorine substitution on cytotoxic potency is about 14-fold (3,9-difluoro being more potent as well as substantially more topo I selective; R/S = 183 vs 9). See ref 3 for details.