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Colchicine Glycorandomization Influences Cytotoxicity and Mechanism of Action

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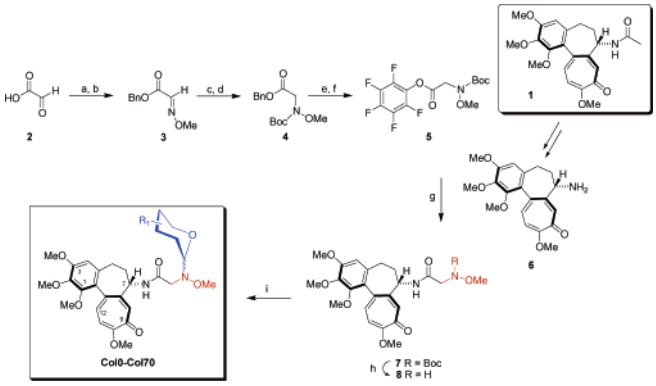
Sugars appended to pharmaceutically important natural products are known to influence drug solubility, pharmacology, target recognition, toxicity, and mechanism of action.[1] However, studies designed to systematically understand and exploit the role of carbohydrates in drug discovery are often limited by the availability of practical synthetic tools. In an attempt to address this issue, we have reported two complementary strategies that allow for the rapid glycosylation of natural product scaffolds.[2] The first (chemoenzymatic glycorandomization) utilizes a set of flexible enzymes (an anomeric kinase, sugar-1-phosphate nucleotidylyltransferase, and natural product glycosyltransferase),[3] while the second (neoglycorandomization) employs a single reaction between a free reducing sugar and a methoxyamine-appended aglycon.[4] While both methods have been successful in glycorandomized library preparation and the identification of compounds with notable activities,[3e,4] applications of glycorandomization to date have been restricted to the natural positions of *O*-glycosylation within natural products.

In an effort to expand upon this work, we set out to assess (i) the potential impact of glycosylation upon natural products that naturally do not contain a carbohydrate moiety and (ii) the utility of extending neoglycosylation to amine-bearing scaffolds. Colchicine (Scheme 1, **1**), the nonglycosylated model for this study, inhibits tubulin polymerization, causing metaphasic mitotic arrest, which leads to rapid cell death.[5] Toxicity limits its clinical use to the treatment of severe inflammatory episodes of gout, familial Mediterranean fever, and Behcet's disease.[6,7] Only two colchicine 3-demethyl-3-glycosides have been reported,[8] and thus, the effects of glycosylation upon this natural product remain largely unknown. Herein we report the synthesis of a 58-member differentially glycosylated colchicine library. Cytotoxicity screens revealed neoglycosylation to modulate the specificity and potency of **1**, and compounds were identified which, unlike **1** (a destabilizer), stabilized tubulin polymerization.

The synthesis of the methoxyamine-tethered aglycon **8** is illustrated in Scheme 1 (eight steps, 40% overall yield). Reaction of glyoxalic acid **2** with methoxyamine followed by benzylation resulted in the formation of methoxyimino acetic acid benzyl ester **3**. Reduction of **3** with borane—pyridine complex followed by Boc protection afforded the intermediate **4**. Reductive debenzylation of **4** and esterification with pentafluorophenol furnished the activated ester-linker **5**. Finally, treatment of **6**[9] with **5** in CH_2Cl_2 followed by deprotection gave the desired methoxyamine-appended aglycon **8**. The chemoselective neoglycosylation reaction of **8** with D-glucose in DMF/AcOH smoothly provided the corresponding colchicine neoglucoside in 65% yield. Consistent with previous reports,[4] the reaction with D-glucose favored the β-isomer (87:13 β/α).[10]

Scheme 1. Synthesis of the Colchicine Neoglycoside Library[a]



[a] Reagents and conditions: (a) $MeONH_2$—HCl, Py, MeOH, room temp, 1 h, 99%; (b) BnBr, $NaHCO_3$, DMF, 70 °C, 16 h, 80%; (c) BH_3—Py, 6 M HCl in EtOH, 15 h, 88%; (d) $(Boc)_2O$, $NaHCO_3$, THF/H_2O (2:1), 16 h, 96%; (e) H_2, Pd/$BaSO_4$, EtOH, 1.5 h, 99%; (f) pentafluorophenol, diisopropyl carbodiimide, CH_2Cl_2/dioxane (1:1) room temp, 16 h, 80%; (g) CH_2Cl_2, 20 h, room temp, 96%; (h) TFA, MeOH, 3 days, 78%; (i) diverse sugars, DMF/AcOH, 24 h, 40 °C, >65%.

Conditions based upon this successful pilot reaction were used in the reaction of 70 unprotected, diverse, free reducing sugars with **8** to give a library of 58 colchicine neoglycosides with yields ranging from 14 to 78% (average overall 51%). All library members were purified, and LC-MS was employed to assess purity (96.2%, average) and confirm identity. Cumulatively, this represents the largest and most diverse glycorandomized library of a nonglycosylated natural product parent scaffold reported to date.

The cytoxicity of the library members was assessed in nine human cancer cell lines representing a broad range of carcinomas including breast, colon, CNS, liver, lung, and ovary and a mouse mammary normal epithelial control cell line. Three standards, **1** (the parent tubulin destabilizer), paclitaxel (a representative tubulin stabilizer), and doxorubicin (a representative tubulin noninteracting cytotoxin), were also examined. All library members displayed IC_{50} values below the "nontoxic" threshold of 10 μM (defined as 3 orders of magnitude greater than the IC_{50} of the parent molecule colchicine) in at least one cell line. Fifteen library members (including **Col6**, **Col19**, **Col21**,[11] **Col45**, **Col56**, and **Col65**, Table 1) displayed IC_{50} values of less than 1 μM in at least one cell line, with some within this subset displaying unique cell line specificities. For example, **Col6** displayed an IC_{50} of 381 nM in SK-OV-3 cells, with potencies in all other cell lines ranging from 691 nM to 1.12 μM. In a similar fashion, **Col45** displayed 403—529 nM potencies in three cell lines (ADR-Res, SF-268, and HCT-116), with decreased potencies (exceeding ~900 nM) in all other cell lines examined. In contrast, the parent **1** displayed a nearly equivalent indiscriminate level of potency in 5 of the 10 cell lines tested, including SK-OV-3 (ranging

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Table 1. Activities of Colchicine Neoglyosides

compound name	compound structure[a]	IC_{50} (Du145)[b]	IC_{50} (HCT-116)[b]	IC_{50} (Hep3B)[b]	IC_{50} (SF-268)[b]	IC_{50} (SK-OV-3)[b]	IC_{50} (ADR-RES)[b]	IC_{50} (NCI-H460)[b]	IC_{50} (A549)[b]	tubulin polymeriz.[c]	synergy (colchicine)[d]	synergy (paclitaxel)[d]
colchicine	1	0.022	0.091	0.329	0.035	0.024	0.027	0.022	0.118	D		++
Col6		0.958	0.792	1.124	0.691	0.381	0.887	0.948	0.942	D	--	+
Col45		0.939	0.529	0.962	0.462	0.887	0.403	1.024	1.055	D	-----	++++
paclitaxel		0.290	0.275	0.166	0.315	0.034	0.043	0.105	0.075	S	+++	
Col19[e]		0.262	0.431	0.437	0.575	0.538	0.315	0.191	0.636	S	+++	-----
Col21[e]		0.294	0.344	1.291	0.349	0.296	0.209	0.355	0.248	S	+	----
doxorubicin		0.339	0.524	0.268	0.385	0.621	0.174	1.001	0.770	no effect	nd	nd
Col56		1.094	0.669	2.228	>1	1.182	0.994	1.094	2.094	no effect	nd	nd
Col65		0.939	0.897	1.875	0.665	1.031	0.694	0.744	2.301	no effect	nd	nd

[a] The saccharide portion of the library member is represented. [b] Cytoxicity (Xb5M-1) as determined by cell titer-glo and calcein AM assays (see Supporting Information for assay parameters). [c] The results of tubulin polymerization assays where "D" designates destabilizer and "S" designates stabilizer (see Supporting Information for assay parameters). [d] Synergism or antagonism in drug combination studies with the parent **1** (a representative destabilizer) or paclitaxel (a representative stabilizer) analyzed via the Chou−Talalay method (see ref 11). The results of synergy assays−legend (combination index): (++++) strong synergism, CI 0.1−0.3; (+++) synergism, CI 0.3−0.7; (++) moderate synergism, CI 0.7−0.85; (+) slight synergism, CI 0.85−0.9; (-) slight antagonism, CI 1.1−1.2; (--) moderate antagonism, CI 1.2−1.45; (---) antagonism, CI 1.45−3.3; (----) strong antagonism, CI 3.3−10. [e] Library member contains both pyranose and furanose forms (see ref 12).

from 22 to 35 nM), as did neoglycosides **Col19** and **Col21**, albeit both were roughly 1 order of magnitude less potent than **1**. It should be noted that, while the best neoglycosides (**Col19** and **Col21**) displayed roughly a 10-fold reduction in potency, the IC_{50} values of these colchicine neoglycosides still fall within the range of the clinically relevant cytotoxins doxorubicin and paclitaxel.

To assess how these structural modifications affect the ability of library members to modulate tubulin polymerization, a fundamental activity of **1**, the same 15 "hits" were submitted to a secondary in vitro tubulin polymerization assay.[13] As expected, eight compounds (including **Col6** and **Col45**, Table 1) exhibited effects on microtubules consistent with the destabilizing effects of **1**. However, three compounds (including **Col56** and **Col65**, Table 1) had no apparent effect on tubulin polymerization (similar to the standard doxorubicin), and surprisingly, two compounds (**Col19** and **Col21**, Table 1) exhibited effects on microtubules consistent with the stabilizing effects of paclitaxel. Drug combination assays were subsequently performed to determine if the mechanism of action of tubulin binding by **1** had been affected for compounds **Col19** and **Col21**.[11] Consistent with the in vitro tubulin polymerization results, **Col19** and **Col21** showed synergism with the parent **1** and antagonism with paclitaxel, suggesting the **Col19**/**21**−tubulin interaction to mirror that of paclitaxel−tubulin. While many synthetic and natural product small molecules are known to stabilize or destabilize tubulin polymerization,[14] to the best of our knowledge, this stands as the first example of interconverting these two distinct mechanisms via simple synthetic derivatization. Cumulatively, this study highlights a simple extension of neoglycorandomization toward amine-bearing scaffolds and illustrates a potential benefit to glycosylating nonglycosylated natural products.

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Supporting Information Available: Experimental procedures, compound/library characterization data, complete cytotoxicity, and tubulin polymerization assay data. This material is available free of charge via the Internet at http://pubs.acs.org.

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