Design and Synthesis of a Taxoid Library Using Radiofrequency Encoded Combinatorial Chemistry

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Radiofrequency encoded combinatorial (REC) chemistry is a recently developed nonchemical encoding strategy in library synthesis. Encoded chemical libraries of complex molecular structures like Taxol can be constructed employing the noninvasive REC strategy and novel solid phase synthesis techniques, as demonstrated by the synthesis of the first 400-membered taxoid library in a discrete format and in quantities of multimilligrams/member.

Taxol[1−3] is a newly approved anticancer agent which is particularly effective against ovarian and breast cancer. Its unique mechanism of action[4] involves promotion of tubulin polymerization and stablization of microtubules, resulting in cell death. However, difficulties related to formulation and multiple drug resistance (MDR) limit the application of Taxol and its close analog, Taxotere, in cancer treatments.[5]

Modification of natural product scaffolds is an attractive strategy toward discovering new and better pharmaceutical agents.[6] The advent of combinatorial chemistry[7] promises to revolutionize traditional drug discovery process by greatly accelerating lead identification and optimization. Despite extensive analog studies in the taxoid field,[3] application of this new technique to the Taxol template has the potential of uncovering new taxoids with improved pharmaceutical properties. In search of such molecules, we applied the recently developed radiofrequency encoded combinatorial (REC) chemistry strategy[8] to the design and synthesis of a taxoid library of general formula **1** (Scheme 1). The criteria used in the molecular design of this library included the following: (a) potential enhancement of water solubility (by incorporation of amide, carboxylic acid, and other polar groups); (b) possible modulation of biological activity (by variation at C-7, C-2′, and C-3′ with a wide range of substituents); and, (c) novel solid phase synthesis. The utilization of microreactors[8a] as the "split & pool" units, rather than the resin beads used in conventional "split & pool" combinatorial synthesis,[9] leads to libraries of discrete compounds in multimilligrams/member quantities without compromising synthesis efficiency as can be the case using parallel synthesis.

Results and Discussion

Attachment of the Taxol Template. As a starting point, the first taxoid library in our plan is the combinatorialization of the Taxol template through the C-2′ and C-7 hydroxy groups and the C-3′ amino group by ester and amide formations. This would not only take advantage of the availability of a vast variety of carboxylic acids as economic and diverse building blocks, but also produce high quality library compounds through well established acylation chemistries. The Initial attempts to link the Taxol template via the C-7 or C-2′ hydroxy groups using the THP ether linker[10] or the related alkoxyethyl ether linker[11] were unsatisfactory due to the high steric hindrance at these sites. A glutamic acid "handle" was then incorporated into the molecule (**1**, Scheme 1), which would provide a readily accessible anchoring point (carboxy group), a well-behaving diversity site (α-amino group), and possible solubility enhancement after cleavage (free carboxylic acid). Even though there has been extensive SAR studies on the modification of the Taxol core at various positions,[3] how the incorporation of this glutamic acid "handle", coupled with modifications at C-7 and C-2′, will actually affect Taxol's biological activities remains unknown or mere speculation until the library is synthesized and evaluated.

Synthesis of the Core Structure 4. The projected solid phase synthesis of the targeted library **1** required the preparation of advanced intermediate **4** (Scheme 2) from commercial baccatin III.[12] Among the reported side-chain coupling strategies, the oxazolidine approach[13] and

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(1) Isolation and structure: Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325−2327.

(2) Total Synthesis: (a) Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E. A.; Paulvannan, K.; Sorensen, E. J. *Nature* **1994**, *367*, 630−634. (b) Hotton, R. A.; Somoza, C.; Kim, H.-B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1597−1600. (c) Masters, J. J.; Link, J. T.; Snyder, L. B.; Young, W. B.; Danishafsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1723−1726.

(3) Reviews: (a) Nicolaou, K. C.; Guy, R. K.; Potier, P. *Sci. Am.* **1996**, *June*, 94−98. (b) Nicolaou, K. C.; Dai, W.-M; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15−44. (c) Kingston, D. G. I.; Molinero, A. A.; Rimoldo, J. M. In *Progress in Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds; Springer: New York, 1993; Vol. 61, pp 1−206.

(4) (a) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665−667. (b) Schiff, P. B.; Horwitz, S. B. *Proc. Nat. Acad. Sci., U.S.A.* **1980**, *77*, 1561−1565.

(5) Landino, L. M.; Macdonald T. L. In *The Chemistry and Pharmacology of Taxol™ and its Derivatives*; Farin, V., Ed.; Elsevier: New York, 1995; Chapter 7.

(6) (a) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press, Inc.: San Diego, 1992. (b) Lavelle, F.; Guéritte-Voegelein, F.; Guénard, D. *Bull. Cancer* **1993**, *80*, 326−331. (c) Klein, L. L. *Tetrahedron Lett.* **1993**, *34*, 2047−2049.

(7) (a) Balkenhohl, F.; Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288−2337. (b) Gordon, E.; Barret, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401.

(8) (a) Nicolaou, K. C.; Xiao, X.-Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2289−2291. (b) Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787−10788.

(9) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84.

(10) Thompson, L. A.; Ellman, J. A. *Tetrahedron Lett.* **1994**, *35*, 9333−9336.

(11) Wang, G. T.; Li, S.; Wideburg, N.; Krafft, G. A.; Kempf, D. J. *J. Med. Chem.* **1995**, *38*, 2995−3002.

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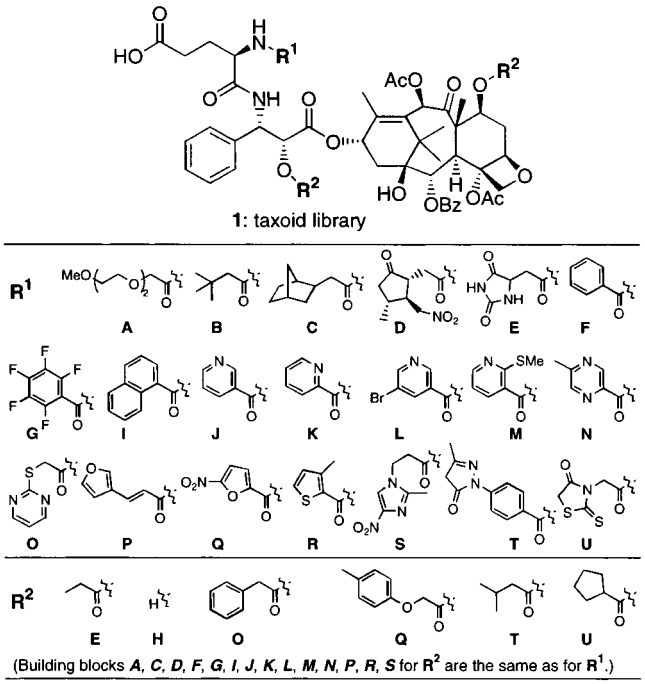
Scheme 1[a]

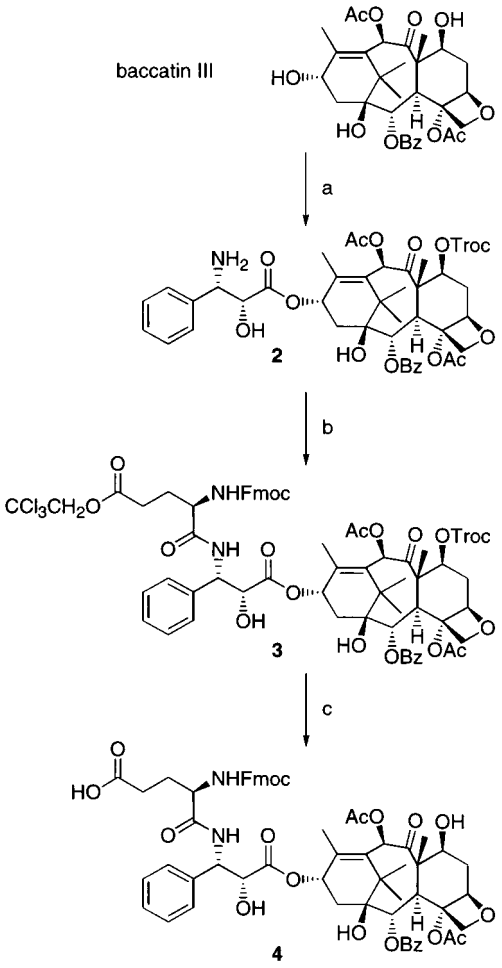


[a]Building blocks used in the taxoid library **1**: 20 × 20 = 400 members. Letters beneath building blocks are codes used in radiofrequency encoding during library synthesis.

the β-lactam approach[14] seem to be promising to yield the desired amino alcohol **2**, and the first approach proved to be fairly successful in our hands. To this end, baccatin III was protected at C-7 as a Troc carbonate,[15] coupled with (4*S*,5*R*)-*N*-Boc-2,2-dimethyl-4-phenyl-5-oxazolidinecarboxylic acid[14,16] under DCC/4-DMAP conditions, and deprotected with formic acid to yield the amino alcohol **2** in 72% overall yield. Subsequent coupling (PyBOP/DIEA) with *N*-Fmoc-*O*-(trichloroethyl)-L-glutamic acid, which was conveniently prepared from *N*-Fmoc-L-glutamic acid *tert*-butyl ester using standard procedures, gave the trichloroethyl ester **3** in 77% isolated yield. Finally, concomitant removal of the Troc group and the trichloroethyl ester group with Zn dust furnished compound **4** in 89% yield.

Library Synthesis. In a standard resin loading procedure, the reagents in solution are usually in large excess in order to drive the loading reaction to completion. Since our Taxol template **4** was derived from the rather expensive and precious baccatin III, a *reversed* (or "*resin trapping*"[17]) loading method was devised to immobilize the template onto solid supports, in which the solid supports are in excess so that complete consumption of the solution reagents can be achieved. Thus, template

Scheme 2[a]



[a](a) Performed according to procedures in references 14−16, three steps, 72% overall; (b) *N*-Fmoc-*O*-(trichloroethyl)glutamic acid, PyBOP, DIEA, CH_2Cl_2, rt, 4 h, 77%; (c) AcOH, MeOH, Zn, 60 °C, 2 h, 89%.

4 was reacted with excess 2-chlorotrityl resin (10 mequiv) via standard trityl ester formation to afford the polymer bound compound **5** (Scheme 3). The loading reaction was conveniently monitored by the disappearance of **4** from the reaction solution using classical solution phase monitoring methods (TLC, HPLC, etc.), and excess trityl chloride was quenched by the addition of methanol.

The loaded resin **5** was then distributed into 400 microreactors[8a] (20 mg each), and the microreactors were subjected to the following reactions and manipulations. First, the microreactors were treated with 5% piperidine/DMF to remove the Fmoc group, yielding amine **6**. The average loading was 2.9 μmol/microreactor as determined by UV measurement of the released 9-methylidenefluorene. The microreactors were then split into 20 equal pools. The reactors in each pool were encoded with a unique rf code (Scheme 1), and each pool was coupled with the corresponding carboxylic acid (R^1, 20 total) under PyBOP/DIEA/DMF conditions to form the amides **7**. The microreactors were re-split into 20 new pools: each new pool was formed by combining one microreactor from each previous pool.[18] The new pools were radiofrequency-encoded accordingly. One pool was set aside (for R^2 = H) and the remaining 19 pools were treated with the second set of carboxylic acids (R^2, 19 total) under DIC/DMAP/CH_2Cl_2 conditions, leading to the resin-bound taxoids **8**. The microreactors were then decoded by

(12) The baccatin III used in this study was from Hauser Laboratories, Chemistry Services, Boulder, CO.

(13) Commerçon, A.; Bézard, D.; Bernard, F.; Bourzat, J. D. *Tetrahedron Lett.* **1992**, *33*, 5185−5188.

(14) (a) Holton, R. A. Eur. Pat. Appl. 1990, EP 400,971. (b) Ojima, I.; Sun, C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. *Tetrahedron Lett.* **1993**, *34*, 4149−4152.

(15) Magri, N. F.; Kingston, D. G. I.; Jitrangsri, C.; Piccariello, T. *J. Org. Chem.* **1986**, *51*, 3239−3242.

(16) Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1991**, *56*, 6939−6942.

(17) During the course of preparing this manuscript, a similar strategy was applied to remove excess reagents in solution phase syntheses: Kaldor, S. W. Presented at 25th National Medicinal Chemistry Symposium of the American Chemical Society, Ann Arbor, MI, June, 1996.

reading the rf codes, distributed into 400 glass vials each labeled with the corresponding rf code, and treated with $AcOH/CF_3CH_2OH/CH_2Cl_2$ (2:1:7, v/v/v) to cleave the final products from the resin in each microreactor, leading to the desired 400-member taxoid library **1**.

The quantity obtained for each compound in the library ranged between 2 to 4 mg, and the purity between 50% to 100% as judged by TLC and HPLC analysis. Confirmation of the structures of 20 randomly-selected members by electrospray MS (Table 1) and ^1H NMR spectroscopy indicated that this synthesis yielded the desired taxoids in the library. Part of the library was purified with HPLC for the purpose of comparing the biological activities of the crude and purified samples.

Conclusion. We have successfully constructed the first 400-member taxoid library **1** by employing the radiofrequency encoded combinatorial (REC) strategy and novel solid phase synthesis techniques. This has demonstrated that using these techniques, complex molecular structures like Taxol can be manipulated on solid supports, and combinatorial libraries of such molecules can be built in a discrete format and in multimilligrams/member quantities. Biological studies of this library and other Taxol derivative libraries are currently in progress and will be reported in due time.

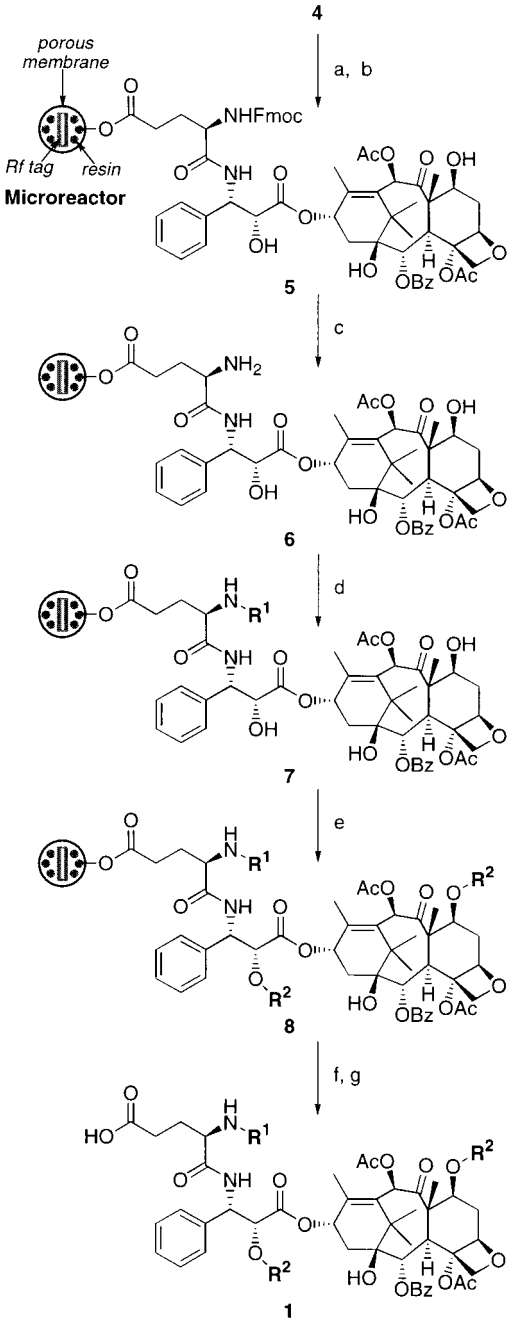
Experimental Section

General Procedures. All moisture sensitive reactions were performed in sealed glass vessels under nitrogen. Reagents and solvents were purchased from either Aldrich or Fisher. Hygroscopic solvents were dried according to standard laboratory procedures prior to use. Chlorotrityl resin (100−200 mesh, 1.05 mmol/g) was purchased from Nova Biochem. All reagents and nonhygroscopic solvents were used as received without further treatment. Thin layer chromatography (TLC) was performed on EM silica gel 60 F_{254} glass plates. Flash column chromatography was performed with EM silica gel 60 (60−120 mesh). Abbreviations: PyBOP, (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophsophate; DIC, 1,3-diisopropylcarbodiimide; TEA, triethylamine; DIEA, diisopropylethylamine; Troc, (2,2,2-trichloroethoxy)carbonyl; rf, radiofrequency.

7-Troc-baccatin III. Baccatin III[12] (1.70 g, 2.90 mmol) was dissolved in anhydrous pyridine (34 mL). Troc-Cl (0.80 mL, 5.81 mmol, 3 equiv) was added. The reaction mixture was stirred at 80 °C for 1 h, cooled to room temperature, diluted with EtOAc (200 mL), washed with water (3 × 100 mL), 10% aqueous $CuSO_4$ (50 mL), water (2 × 100 mL), and brine (50 mL), and dried over $MgSO_4$. The $MgSO_4$ was filtered off, and the solution was concentrated under vacuum. Flash column chromatography of the residue on silica gel with 20−40% EtOAc/petroleum ether yielded a white solid (1.71 g, 77%): R_f = 0.75 (silica, 80% EtOAc in petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 1.09 (s, 3 H), 1.14 (s, 3 H), 1.82 (s, 3 H), 1.97−2.10 (m, 2 H), 2.13 (s, 3 H), 2.17 (s, 3H), 2.20−2.29 (m, 2 H), 2.30 (s, 3H), 2.60−2.68 (m, 1 H), 4.02 (d, J = 6.9 Hz, 1 H), 4.15 (d, J = 8.5 Hz, 1 H), 4.33 (d, J = 8.5 Hz, 1 H), 4.65 (d, J = 12.0 Hz, 1 H), 4.88 (br t, J = 8.0 Hz, 1 H), 5.00 (d, J = 9.1 Hz, 1 H), 5.04 (d, J = 12.0 Hz, 1 H), 5.58−5.65 (m, 2 H), 6.39 (s, 1 H), 7.47−7.52 (m, 2 H), 7.62 (t, J =7.4, 1 H), 8.10 (d, J = 7.8 Hz, 2 H); LRMS (electrospray) m/z $[M + Na]^+$ 785, calcd for $C_{34}H_{39}Cl_3O_{13}Na$ 785.

(18) The split of microreactors for the second step is a *nonstatistical* redistribution since the ratio between the number of reactors and the library size is 1:1 (i.e. *zero redundancy*), and statistical split cannot be used. Apparently, this method is applicable only in the second step of a synthesis and only when the reactors are not pooled together after the first step. A similar split strategy, called *directed sorting*, which does *not* have these limitations, has been recently reported by us: Xiao, X. Y.; Zhao, C.; Potash, H.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 780−782.

Scheme 3[a]



[a](a) Excess 2-chlorotrityl resin (8.0 g, 10 m equiv), DIEA, CH_2Cl_2, rt, 3 h, then MeOH, rt, 0.5 h; (b) resin was distributed into 400 microreactors; (c) 5% piperidine in DMF, rt, 0.5 h; (d) microreactors were split into 20 equal pools, and each pool was treated with carboxylic acid (R^1), DIEA, PyBOP, DMF, rt, 4 h; (e) microreactors were resplit into 20 new pools (see main text), and each pool was treated with carboxylic acids (R^2), DIC, DMAP, CH_2Cl_2, rt, 48 h; (f) microreactors were decoded and distributed into 400 glass vials; (g) AcOH, CH_2Cl_2, CF_3CH_2OH, rt, 4.

7-Troc-13-*O*-(*N*,*O*-ethyleidene-*β*-phenylisoserinyl)baccatin III. The preceding compound (1.71 g, 2.24 mmol) was dissolved in 70 mL toluene. (4*S*,5*R*)-*N*-Boc-2,2-dimethyl-4-phenyl-5-oxazolidinecarboxylic acid[14,16] (1.08 g, 3.36 mmol, 1.5 equiv), 4-DMAP (137 mg, 1.12 mmol, 0.5 eq.), and DCC (0.74 g, 3.59 mmol, 1.6 equiv) were added sequentially. The reaction mixture was heated at 80 °C for 2 h, cooled to room temperature, diluted with EtOAc/diethyl ether (1:1, 200 mL), and washed with 10% $CuSO_4$ (2 × 80 mL), water (2 × 80 mL), and brine (80 mL). The solution was dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude product was purified by column chromatography on silica gel with 15−30%

Table 1. Structures and Analytical Data of 20 Members from the 400-Membered Taxoid Library 1

entry	Rf code[a]	R^1	R^2	*m/z*[b]	crude yield (%)[c]	purified yield (%)[d]
1	*II*			1340 (1341)	100 (55)	39
2	*IR*			1279 (1280)	83 (87)	50
3	*MO*			1266 (1265)	74 (100)	40
4	*PU*			1192 (1191)	81 (100)	72
5	*RP*			1241 (1242)	75 (84)	35
6	*BU*			1268 (1269)	86 (100)	45
7	*CC*			1288 (1287)	86 (100)	72
8	*FP*			1221 (1222)	107 (93)	58
9	*KO*			1218 (1219)	105 (85)	35
10	*MR*			1276 (1277)	81 (100)	45
11	*PC*			1272 (1271)	65 (100)	34
12	*IJ*			1243 (1242)	83 (87)	64
13	*RM*			1303 (1304)	61 (100)	28
14	*PK*			1209 (1208)	80 (85)	27
15	*MM*			1332 (1331)	80 (100)	70
16	*RF*			1211 (1210)	74 (79)	27
17	*MC*			1303 (1302)	90 (71)	33
18	*FA*			1302 (1303)	71 (94)	38
19	*BF*			1183 (1184)	78 (82)	60
20	*CM*			1317 (1316)	68 (100)	44

[a] Codes (in the format of "$\mathbf{R^1R^2}$") assigned to building blocks during library synthesis (See Scheme 1). [b] *m/z* refers to $[M + H]^+$ (electrospray MS) or $[M - H]^-$ (for entries 1, 2, 5, 6, 8, 9, 10, 13, 18, and 19). Calculated values are given in parentheses. [c] Yields are based on initial loading of 2.9 μmol per microreactor (see main text). Product purity was estimated by HPLC analysis and given in parentheses. HPLC conditions: HP 1050 with an HP ODS Hypersil C-18 column (5 μm, 100 × 4.6 mm); gradients: 75% acetonitrile/water to 100% acetonitrile in 10 min; detection at 254 nm. [d] Crude samples were purified with preparative HPLC using similar gradients to that in note *c*.

EtOAc/petroleum ether to yield 2.23 g of the acetonide as a white solid (93%): $R_f = 0.85$, 50% EtOAc in petroleum ether; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.10 (br s, 9 H), 1.16 (s, 3 H), 1.24 (s, 3 H), 1.76 (s, 3 H), 1.80 (s, 3 H), 1.92 (s, 3 H), 2.00 (s, 3 H), 2.17 (s, 6 H), 1.50−2.20 (m, 4 H), 2.55−2.70 (m, 1 H), 3.93 (d, $J = 7.0$ Hz, 1 H), 4.11 (d, $J = 8.5$ Hz, 1 H), 4.27 (d, $J = 8.5$ Hz, 1 H), 4.47 (d, $J = 6.6$ Hz, 1 H), 6.64 (d, $J = 12.0$ Hz, 1 H), 4.91 (d, $J = 9.0$ Hz, 1 H), 5.03 (d, $J = 12.0$ Hz, 1 H), 5.58 (dd, $J = 7.1$ Hz, $J = 10.8$ Hz, 1 H), 5.65 (d, $J = 7.0$ Hz, 1 H), 6.26 (t, $J = 9.1$ Hz, 1 H), 6.36 (s, 1 H), 7.32−7.43 (m, 5 H), 7.48−7.53 (m, 2 H), 7.64 (t, $J = 7.3$ Hz, 1 H), 8.04 (d, $J = 7.5$ Hz, 1 H); LRMS (electrospray) *m/z* $[M + Na]^+$ 1088, calcd for $C_{51}H_{60}Cl_3NO_{17}Na$ 1088.

7-Troc-13-*O*-[*N*-[*N*-Fmoc-*O*-(trichloroethyl)glutamyl]-*β*-phenylisoserinyl]baccatin III (3). The above acetonide (2.10 g, 1.97 mmol) was dissolved in cold formic acid (170 mL, 0 °C) and stirred at 0 °C for 3 h. The reaction mixture was concentrated at room temperature under vacuum. The residue was redissolved in EtOAc (200 mL), washed with saturated $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated under vacuum to yield 1.81 g of the amino alcohol **2** as a white solid (quantitative): $R_f = 0.42$, 5% MeOH in CH_2Cl_2.

N-Fmoc-*O*-(trichloroethyl)glutamic acid (1.25 g, 2.50 mmol, 1.2 equiv), prepared from *N*-Fmoc-glutamic acid *tert*-butyl ester using standard procedures, was dissolved in 50 mL of CH_2Cl_2. DIEA (0.55 mL, 3.2 mmol, 1.5 equiv) and PyBOP (1.19 g, 2.29 mmol, 1.1 equiv) were added sequentially. The colorless solution was stirred at room temperature for 20 min, followed by addition of amino alcohol **2** (1.93 g, 2.08 mmol, in 100 mL of CH_2Cl_2). The reaction mixture was stirred at room temperature for 4 h, diluted with EtOAc (500 mL), and washed with saturated phosphate buffer (pH = 4, 3 × 200 mL), saturated $NaHCO_3$ (3 × 200 mL), and brine (200 mL). The solution was dried over $MgSO_4$ and concentrated under vacuum. The residue was flash chromatographed on silica gel with 0−10% EtOAc/CH_2Cl_2 to yield the desired ester **3** as a white solid (2.25 g, 77%): $R_f = 0.55$, 5% MeOH in CH_2Cl_2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.18 (s, 3 H), 1.25 (s, 3 H), 1.84 (s, 3 H), 1.86 (s, 3 H), 2.17 (s, 3 H), 2.30 (d, $J = 8$,7 Hz, 2 H), 2.38 (s, 3 H), 1.5−2,8 (m, 9 H), 3.92 (d, $J = 6.8$ Hz, 1 H), 4.17−4.30 (m, 3 H), 4.31 (d, $J = 8.6$ Hz, 1 H), 4.31−4.45 (m, 2 H), 4.63 (d, $J = 12.0$ Hz, 1 H), 4.68 (br d, $J = 19.6$ Hz, 2 H), 4.94 (d, $J = 9.0$ Hz, 1 H), 5.01 (d, $J = 12.0$ Hz, 1 H), 5.45−5.55 (m, 2 H), 5.69 (d, $J = 6.8$ Hz, 1 H), 6.20−6.28 (m, 1 H), 6.34 (s, 1 H), 7.05−7.60 (m, 16 H), 7.75 (d, $J = 7.4$ Hz, 1 H), 8.11 (d, $J = 7.6$ Hz, 1 H); LRMS (electrospray) *m/z* $[M + H]^+$ 1405, calcd for $C_{65}H_{67}Cl_6N_2O_{20}$ 1405.

13-[*N*-(*N*-Fmoc-glutamyl)-*β*-phenylisoserinyl]baccatin III (4). Trichloroethyl ester **3** (2.23 g, 1.58 mmol) was dissolved in MeOH/AcOH (4:3, v/v, 87 mL). Activated zinc powder (4.2 g, large excess) was added carefully. The reaction mixture was vigorously stirred at 60 °C for 2 h, cooled to room temperature, and filtered through a glass wool pad. The clear solution was concentrated under vacuum, and the residue was redissolved in a mixture of EtOAc and water (1:1, 500 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The organic solutions were combined, dried over $MgSO_4$, and concentrated under vacuum. Flash column chromatography on silica gel with 5% MeOH/CH_2Cl_2 to 0.1% AcOH/5% MeOH/CH_2Cl_2 yielded the desired carboxylic acid **4** as a white solid (1.53 g, 89%): $R_f = 0.40$, 5% MeOH and 0.1% AcOH in CH_2Cl_2 ; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (s, 3 H), 1.26 (s, 3 H), 1.67 (s, 3 H), 1.80 (s, 3 H), 2.24 (s, 3 H), 2.32 (s, 3 H), 1.50−2.50 (m, 8 H), 2.51−2.52 (m, 1 H), 3.71−3.80 (m, 1 H), 4.15 (t, $J = 7.0$ Hz, 1 H), 4.20 (d, $J = 8.5$ Hz, 1 H), 4.28 (d, $J = 8.5$ Hz, 1 H), 4.34 (d, $J = 7.0$ Hz, 1 H), 4.36−4.50 (m, 2 H), 4.65 (d, $J = 2.5$ Hz, 1 H), 4.93 (d, $J = 9.1$ Hz, 1 H), 5.50−5.53 (m, 1 H), 5.62 (d, $J = 8.2$ Hz, 1 H), 5.67 (d, $J = 7.1$ Hz, 1 H), 6.26 (s, 1 H), 7.20−7.60 (m, 16 H), 7.74 (d, $J = 7.4$ Hz, 1 H), 8.11 (d, $J = 7.5$ Hz, 1 H); LRMS (electrospray) *m/z* $[M + Na]^+$ 1123, calcd for $C_{60}H_{64}N_2O_{18}Na$ 1123.

Resin 5. To a solution of carboxylic acid **4** (1.44 g, 1.31 mmol) and DIEA (4.4 mL, 25.2 mmol, 0.5 M final concentration) in anhydrous CH_2Cl_2 (50 mL) at room temperature was added 2-chlorotrityl resin (8.00 g, 1.6 mmol/g, 10 m equiv) in small portions with constant stirring. The reaction vessel was sealed and shaken at room temperature for 3 h. The yellow resin gradually turned into deep purple. TLC analysis of the reaction solution showed that carboxylic acid **4** was completely consumed. MeOH (8.4 mL) was then added, and the reaction was shaken at room temperature for 30 min. The solution was filtered, and the purple resin was washed using the following *standard washing procedure*: MeOH (containing 0.5% TEA) and CH_2Cl_2 (containing 0.5% TEA) alternately for a total of four cycles. The resin was dried under vacuum at room temperature overnight (9.5 g).

Resin 6. Resin **5** was distributed into 400 microreactors[8a] (20 mg each). The microreactors were then immersed in 5% piperidine/DMF (400 mL) and shaken at room temperature for 30 min. Aliquots was removed and measured by UV at 302 nm, and the loading was determined to be 2.9 μmol/

microreactor. The microreactors were washed thoroughly according to the standard washing procedure and dried under vacuum at room temperature for overnight.

Resin 7. The above microreactors were split into 20 groups each containing 20 microreactors. Each group was encoded with a unique rf code (Scheme 1) and subjected to coupling with the corresponding carboxylic acid (R^1, Scheme 1) under the following conditions: carboxylic acid (0.1 M), DIEA (0.2 M), PyBOP (0.1 M), DMF (20 mL), room temperature, 2 h; additional carboxylic acid and PyBOP (half the previous amounts), room temperature, 2 h. The reactions were quenched with methanol. The 20 groups of microreactors were kept separate, washed according to the standard procedure, and dried under vacuum at room temperature overnight to yield the resin-bound amides **7**.

Resin 8. The above microreactors were resplit into 20 new groups by combining one microreactor from each of the previous groups. Each of the 20 new groups was encoded with a unique rf code. One group, corresponding to R^2 = H, was set aside. Each of the remaining 19 groups was then subjected to coupling with one of the second set of carboxylic acids (R^2, Scheme 1) under the following conditions: carboxylic acid (0.4 M), 4-DMAP (0.35 M), DIC (0.4 M), CH_2Cl_2 (20 mL), room temperature, 24 h; additional carboxylic acid and DIC (half the previous amounts), room temperature, 24 h. The reactions were quenched by the addition of methanol. The microreactors were pooled together, washed, and dried under vacuum to afford the desired resin-bound taxoids **8**.

Taxoid Library 1. The above 400 microreactors were distributed into 400 glass vials (8 mL capacity). The rf code in each Microreactor was read, and the vial was labeled with that code. $AcOH/CF_3CH_2OH/CH_2Cl_2$ (2:1:7, 2 mL) was then added into each vial. The vials were sealed and shaken at room temperature for 4 h. The microreactors were taken out and rinsed with methanol (1 mL/microreactor) and CH_2Cl_2 1 mL/microreactor). The solutions in the vials were then slowly evaporated in a SpeedVac under carefully controlled vacuum. The residues were then redissolved in benzene (2 mL/vial), frozen, and lyophilized to yield 400 powders with colors ranging from white to brown. Part of the library (20 compounds) were analyzed by mass spectrometry and/or ^{1}H NMR spectroscopy, and the structures were confirmed to be the desired. Selective ^{1}H NMR data (see Table 1 for purity. Compounds are named using the rf codes in Scheme 1 in the format of "**1-R^{1}R$^{2\prime\prime}$**"):

1-BU. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, J = 8.02 Hz, 2 H), 7.87−7.85 (m, 1 H), 7.62−7.50 (m, 2 H), 7.53−7.7.46 (m, 2 H), 7.40−7.25 (m, 4 H), 6.40 (d, J = 7.5 Hz, 1 H), 6.32 (s, 1 H), 6.19 (m, 1 H), 5.70−5.68 (m, 2 H), 5.70−5.60 (m, 2 H), 5.53 (dd, J = 7.2 Hz, J = 10.3 Hz, 1 H), 5.30 (d, J = 3.8 Hz, 1 H), 4.94 (br d, J = 9.3 Hz, 1 H), 4.54−4.38 (m, 1 H), 4.29 (d, J = 8.4 Hz, 1 H), 4.18 (d, J = 8.4 Hz, 1 H), 3.91 (d, J = 7.0 Hz, 1 H), 2.80−0.80 (m, 29 H), 2.42 (s, 3 H), 2.13 (s, 3 H), 2.08 (s, 3 H), 1.97 (s, 3 H), 1.21 (s, 3 H), 1.15 (s, 3 H), 0.94 (s, 9 H).

1-IR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25−8.16 (m, 1 H), 8.10−8.03 (m, 2 H), 7.90 (d, J = 8.3 Hz, 1 H), 7.84 (d, J = 8.3 Hz, 1 H), 7.60−7.30 (m, 15 H), 6.88−6.85 (m, 2 H), 6.44 (s, 1 H), 6.30−6.27 (m, 1 H), 5.84 (dd, J = 3.2 Hz, J = 9.5 Hz, H), 5.75−5.65 (m, 2 H), 5.47 (d, J = 3.6 Hz, 1 H), 5.00 (br d, J = 9.3 Hz, 1 H), 4.90−4.85 (m, 1 H), 4.30 (d, J = 8.4 Hz, 1 H), 4.21 (d, J = 8.4 Hz, 1 H), 4.01 (d, J = 7.1 Hz, 1 H), 2.80−2.72 (m, 1 H), 2.60−1.20 (m, 8 H), 2.53 (s, 3 H), 2.45 (s, 3 H), 2.43 (s, 3 H), 2.08 (s, 3 H), 2.01 (s, 3 H), 1.90 (s, 3 H), 1.46 (s, 3 H), 1.25 (s, 3 H).

1-RF. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, J = 7.6 Hz, 2 H), 8.05 (d, J = 7.6 Hz, 2 H), 7.92 (d, J = 7.6 Hz, 2 H), 7.65−7.20 (m, 15 H), 6.90 (d, J = 4.9 Hz, 1 H), 6.55 (br d, J = 7.3 Hz, 1 H), 6.40 (s, 1 H), 6.32−6.28 (m, 1 H), 5.82 (dd, J = 2.5 Hz, J = 9.5 Hz, 1 H), 5.72−5.67 (m, 2 H), 5.00 (br d, J = 8.5 Hz, 1 H), 4.80−4.76 (m, 1 H), 4.30 (d, J = 8.3 Hz, 1 H), 4.21 (d, J = 8.3 Hz, 1 H), 4.00 (d, J = 7.0 Hz,1 H), 2.85−2.75 (m, 1 H), 2.55−1.00 (m, 8 H), 2.53 (s, 3 H), 2.38 (s, 3 H), 2.09 (s, 3 H), 2.03 (s, 3 H), 1.96 (s, 3 H), 1.48 (s, 3 H), 1.47 (s, 3 H).

1-MO. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (d, J = 4.5 Hz, 1 H), 8.07 (d, J = 7.5 Hz, 2 H), 7.69 (d, J = 7.5 Hz, 1 H), 7.58−7.00 (m, 19 H), 6.28 (s, 1 H), 6.17−6.12 (m, 1 H), 5.68−5.60 (m, 2 H), 5.56 (dd, J = 7.2 Hz, J = 10.4 Hz, 1 H), 5.35 (d, J = 4.3 Hz,1 H), 4.87 (br d, J = 9.5 Hz, 1 H), 4.67−4.62 (m, 1 H), 4.26 (d, J = 8.4 Hz,1 H), 4.14 (d, J = 8.4 Hz, 1 H), 3.89 (d, J = 6.9 Hz, 1 H), 3.70−3.60 (m, 5 H), 2.60−1.00 (m, 9 H), 2.51 (s, 3 H), 2.40 (s, 3 H), 2.19 (s, 3 H), 2.08 (s, 3 H), 1.93 (s, 3 H), 1.47 (s, 3 H), 1.46 (s, 3 H).

1-PK. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (br d, J = 4.4 Hz, 1 H), 8.76−8.72 (m, 2 H), 8.64 (d, J = 4.5 Hz, 1 H), 8.23 (d, J = 7.7 Hz, 1 H), 8.16 (d, J = 7.5 Hz, 2 H), 7.92−7.70 (m, 6 H), 7.60−7.20 (m, 10 H), 6.70−6.60 (m, 1 H), 6.44 (br s, 1 H), 6.36 (s, 1 H), 6.32−6.28 (m, 1 H), 6.08 (d, J = 15.5 Hz, 1 H), 5.91 (dd, J = 1.7 Hz, J = 9.3 Hz, 1 H), 5.86 (dd, J = 7.3 Hz, J = 10.3 Hz, 1 H), 5.72−5.68 (m, 2 H), 5.02 (d, J = 8.9 Hz, 1 H), 4.73−4.66 (m, 1 H), 4.34 (d, J = 8.4 Hz, 1 H), 4.26 (d, J = 8.4 Hz, 1 H), 4.06 (d, J = 7.0 Hz, 1 H), 2.85−2.74 (m, 1 H), 2.60−1.10 (m, 8 H), 2.49 (s, 3 H), 2.12 (s, 3 H), 2.03 (s, 3 H), 1.97 (s, 3 H), 1.47 (s, 3 H), 1.46 (s, 3 H).

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Supporting Information Available: ^{1}H NMR spectra for intermediates (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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