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Combinatorial Modification of Natural Products: Preparation of Unencoded and Encoded Libraries of *Rauwolfia* Alkaloids

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Abstract—We report the preparation of combinatorial libraries which consist of derivatives of the stereoisomeric alkaloids yohimbine and rauwolscine–members of the *Rauwolfia* genus. The chemistry was performed on solid support using the divide-and-pool method, and involved the derivatization of the E-ring carboxylates and hydroxyls of these alkaloids with 36 amino acids and 22 carboxylic acids, respectively, to afford 792 bifunctionalized derivatives. The rauwolscine library was prepared using an encoding strategy in which the identity of each incorporated amino acid was recorded by cosynthesizing chemically inert tags prior to the pooling step. The general strategy for library synthesis exploits existing functionality present on the natural products, and should be applicable to other families of secondary metabolites. Copyright © 1996 Elsevier Science Ltd

Introduction

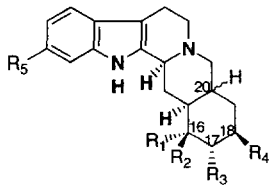
We report herein a strategy for generating molecular diversity which utilizes natural products as template molecules for combinatorial modification. Combinatorial chemistry has emerged as a powerful tool for the assembly of large collections of synthetic molecules, and has been embraced by the pharmaceutical industry as a potential source of biologically active lead compounds.[1] Historically, however, the most prolific source of lead compounds and novel pharmacophores has been natural products — the extremely diverse collections of secondary metabolites produced by plant, marine, and microorganisms as a result of millions of years of evolution. While as few as 35,000 known, fully characterized natural products have been reported,[2] natural products or their derivatives are estimated to account for half of all prescription drugs in industrialized countries.[3] The combinatorial modification of natural products should enable ready access to libraries of this rich source of biologically active compounds.

There are numerous examples of the modification of natural products to afford derivatives with altered biological activities or improved pharmacokinetics.[4] For example, cephalosporin C is not a clinically useful antibiotic; however, the discovery of an efficient method for hydrolysing its exocyclic amide allowed the synthesis of other amide derivatives which have improved potency, spectrum, and pharmacokinetics (e.g. cephalothin and cephalexin).[4] The analgesic codeine and the opiate antagonist nalorphine are simple analogues of morphine, while the hypotensive agent metocurine is a methylation product of tubocurarine.[4] For our initial approach to the combinatorial modification of natural products, we modified the E-ring carboxylates and hydroxyls of the *Rauwolfia* alkaloids yohimbine (**1a**) and rauwolscine (**2a**) (Fig. 1). Some of this work was performed using an encoding strategy in which chemically inert molecular tags were cosynthesized with each natural product derivative, thus permiting identification of biologically active members of the library by simply decoding the associated tags.

Results and Discussion

Selection of starting materials

There are over 50 known, characterized alkaloids which belong to the genus *Rauwolfia*, many of which



	R_1	R_2	R_3	R_4	R_5	C20
(1) Yohimbine	XO_2C-	H	OH	H	H	β
(2) Rauwolscine	H	XO_2C-	OH	H	H	α
(3) Corynanthine	H	XO_2C-	OH	H	H	β
(4) Reserpine	XO_2C-	H	OMe		OMe	α

a: X = CH_3
b: X = H

Figure 1. *Rauwolfia* alkaloids which were examined for their ease of modification on a solid support.

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are commercially available.[5] The indole alkaloids yohimbine (**1a**), rauwolscine (**2a**), corynanthine (**3a**), and reserpine (**4a**) were selected for initial study (Fig. 1). These compounds have been shown to have activity as antihypertensive agents, anti-arrythmics, and as adrenoceptor antagonists; however, the activity of the parent alkaloids was not a primary concern, as the libraries of these compounds were intended to enter general high-throughput screens.[5] Their selection satisfied several important design criteria. First, they were available in multigram quantities, which ensured that there would be enough starting material for exploring general methods for their modification on solid supports as well as for subsequent library preparation. Second, the compounds needed to have a convenient functional group to serve as a point of attachment to the solid support, and it was hoped that the conversion of the E-ring esters to carboxylic acids would provide this requisite functionality. A further requirement was the presence of functionality which could be exploited for synthetic modification, and the indole nitrogen, *beta*-carboline nitrogen, and the E-ring carboxylate and hydroxyl were attractive in this regard. Most importantly, the compounds needed to be stable to the conditions required for solid-phase chemistry, including the reagents and conditions used for cleaving them from the support.

To determine whether these natural products were suitable for modification on solid support, we examined their ease of saponification, the conditions required to attach them to a solid support, and their stability to cleavage conditions such as trifluoroacetic acid (TFA; acid-cleavable linkers) or photolysis (photocleavable linkers). Saponification was performed in methanolic potassium hydroxide for 2 h at reflux.[6] Reaction mixtures were concentrated, diluted with water and then acidified with concd HCl to precipitate the alkaloids as their hydrochloride salts.[6] A four-fold excess of each free acid was then coupled to TentaGel-S-NH_2 resin functionalized with the TFA-labile Rink amide linker (RAM). A variety of carboxylic acid activating agents were explored, and HATU activation in the presence of DIEA was found to be superior. The alkaloids were cleaved from the support by treatment with TFA for 2 h, the filtrates collected, concentrated, and analyzed by HPLC, MS, and ^{1}H NMR spectroscopy (data not shown). In addition, each compound was tested for its stability to conditions required for cleavage from solid supports functionalized with a photocleavable linker.[7] Aliquots of each alkaloid (2.8 mM in DMSO) were placed in open-face scintillation vials (1–1.5 cm pathlength) and irradiated from above with 10 mW/cm^2 of 365 nm UV light (mercury arc lamp, 350–450 nm dichroic reflector) for 2 h, with portions removed every 30 min and examined by reverse-phase HPLC.

Poor results were obtained in the analysis of Reserpic acid (**4b**; R_4=OH). The hydroxyl at C-18 of this alkaloid was protected as its fluorenylmethyloxycarbonyl (Fmoc) carbonate to prevent it from polymerizing once the carboxylic acid was activated, and this compound was used to acylate several solid supports under a variety of conditions. Coupling efficiencies as determined by spectrophotometrically quantifying Fmoc release from the acylated resins were low. In addition, cleaving these resins with TFA yielded no discernable products, although the presence of the alkaloid on the solid support was confirmed by gel-phase magic angle ^{1}H NMR (data not shown).[8] The methoxy-substituted indole of reserpic acid is electron rich, and it is possible that this ring may have been alkylated by the acid-generated cation of the cleavable linker, but this is unconfirmed (note that the other alkaloids in Figure 1 contain unsubstituted indole rings). A photocleavable linker[7] was explored as an orthogonal alternative; however, reserpic acid was found to degrade to uncharacterized products upon photolysis using the conditions described above, and this alkaloid was not selected for further study. During the above analysis it was also found that a stereocenter in corynanthine (**3a**) inverted upon saponification to afford yohimbinic acid (**1b**).[9] We are currently exploring methods for preparing the free acid without inversion, but this alkaloid was not used further in the work described herein.

Chemistry

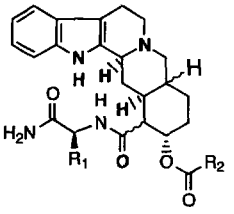
Yohimbinic acid (**1b**) and rauwolscinic acid (**2b**) were selected for combinatorial library preparation. Much of the prior work on this family of compounds has focussed on modifications of the E-ring carboxylate and hydroxyl in efforts to obtain derivatives with altered biological properties, and esterification of these functional groups is the most common modification.[5,6] The first approach toward their chemical modification on solid phase is outlined in Scheme 1. TentaGel-S-NH_2 RAM resin in a glass reaction vessel containing a fritted glass filter was first deprotected with piperidine, washed, and then acylated with an appropriately side-chain protected Fmoc-amino acid using HATU activation in the presence of DIEA. This was deprotected with piperidine and the liberated amine acylated with a four-fold excess of the alkaloid of interest using HATU activation. Protection of the E-ring hydroxyl during this step was found to be unnecessary, as polymerization products were not observed for either compound. Subsequent acylation of the hydroxyl at C-17 was optimized using yohimbinic acid, because the axial conformation it adopts in this alkaloid is thought to hinder esterification.[6a] Acylation was attempted using a variety of conditions, but was found to proceed most smoothly via a large excess of the symmetrical anhydride of the desired carboxylic acid. Symmetrical anhydrides were prepared using 0.5 equiv DIC in dichloromethane. After 30 min the resulting anhydride was added to the resin in one portion and the acylation allowed to proceed for 2 h in the presence of DIEA and DMAP. After a second acylation ('double coupling'), the resin was washed and the desired alkaloid derivative cleaved from the support by treatment with TFA for 2 h. Virtually every amino acid examined was readily incorporated into the above

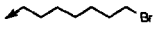
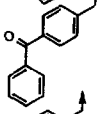
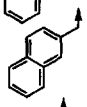
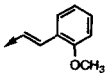
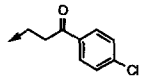
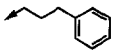
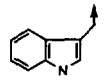
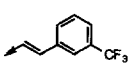
synthetic scheme in high yield; however, over 50 different carboxylic acids were screened to identify 22 which afforded crude yields of acylated alkaloids in excess of 60% as estimated by reverse-phase HPLC. Representative compounds prepared in this fashion are outlined in Table 1. Isolated yields for the three-step procedure were moderate to good.

Unencoded library preparation: yohimbinic acid

A library of yohimbinic acid (**1b**) derivatives was similarly prepared from 36 amino acid[10] and 22 carboxylic acid[11] 'building blocks' using the divide-and-pool method of Furka.[12] First, the 36 natural and unnatural amino acids were individually coupled to TentaGel-S-NH_2 resin as described above. The resins were pooled, deprotected with piperidine, and then acylated with yohimbinic acid to afford a large bead mass consisting of 36 yohimbamides. This was portioned into 22 separate reaction vessels, and each bead mass was acylated with an excess of a single carboxylic acid activated as its symmetrical anhydride. After a second acylation, the resins were washed and then cleaved with TFA to afford 22 pools of 36 compounds — a library of 792 difunctionalized yohimbamides. MS analysis (flow injection ESI) of the pool acylated with the sterically hindered reagent isobutyric anhydride revealed molecular ions for 34 out of the possible 36 desired compounds (data not shown).

Table 1. Representative functionalized *Rauwolfia* alkaloids synthesized on solid support



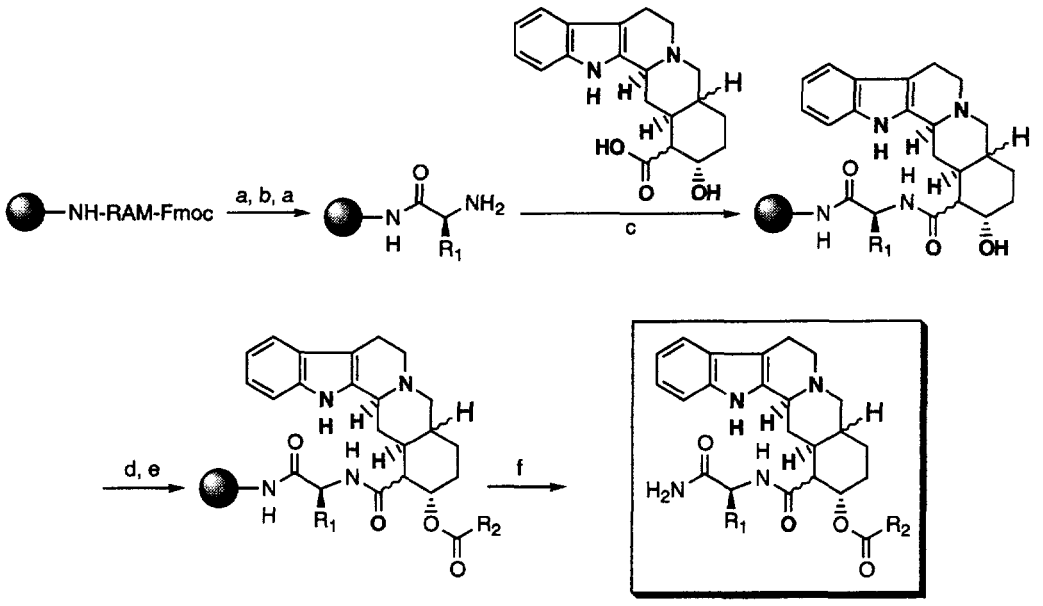
	R_1	R_2	Alkaloid	Isolated yield (%)
5			**1b**	56
6				45
7				37
8				47
9				42
10				65
11				39
12			**2b**	51
13				47
14				51
15				48
16		CH_3		28
17				13

Encoded library preparation: rauwolscinic acid

Encoding procedure. A rauwolscinic acid (**2b**) library was also prepared as described above; however, this work was performed using an 'encoding' strategy.[13–15] Encoding is a technique in which easily detectable surrogate analytes, or 'tags', are cosynthesized with the desired ligands. Upon biological assay of the often subanalytical amounts of compound present on single beads, structural elucidation is achieved by decoding the associated tags. The encoding methodology employed here uses chemically robust secondary amines as tags, and these amines are incorporated into a *N*-(dialkylcarbamoylmethyl)-glycine coding oligomer through simple amide bond chemistry (Scheme 2).[15] In the decoding process, acidic hydrolysis of the tagging polymer regenerates the secondary amines. These are derivatized with the reporter molecule dansyl chloride, and the resulting *N,N*-dialkylsulfonamides are easily distinguished at sub-picomole levels by reverse-phase HPLC with fluorescence detection.[15]

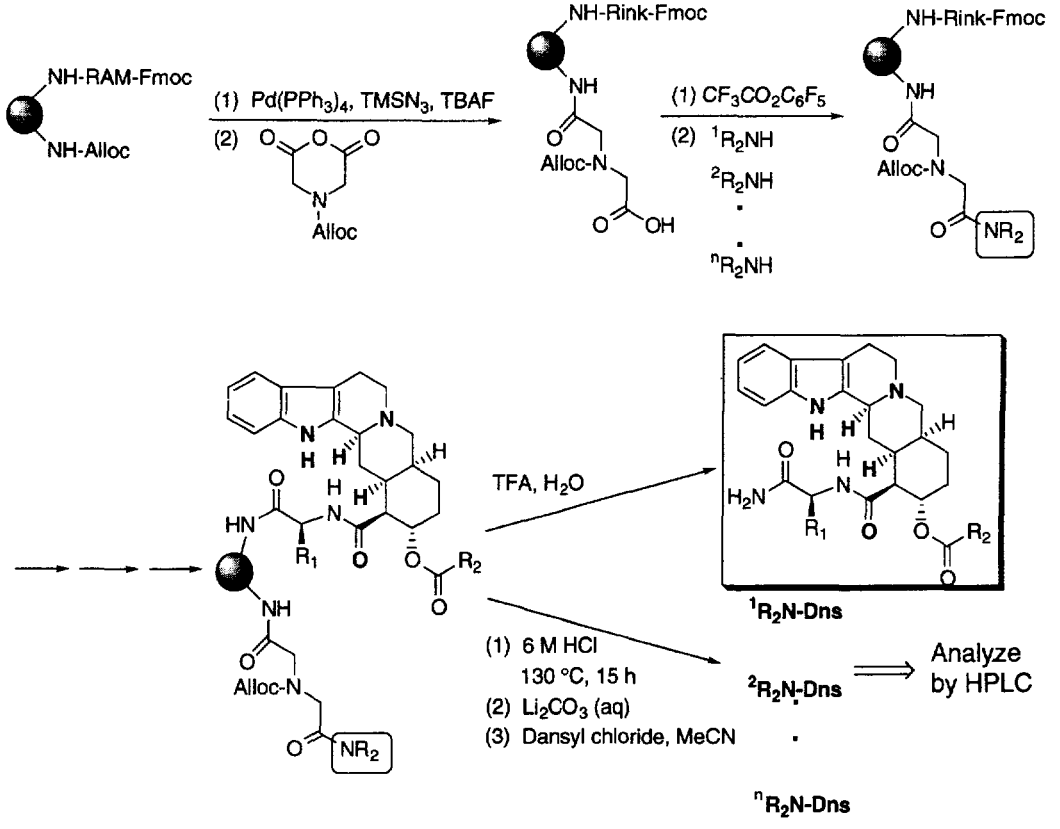
Preparation of encoded library

An encoded library of acylated rauwolscinamides was prepared on 130 mm TentaGel-S-NH_2 as described in Scheme 2. First, the beads were orthogonally differentiated by acylation with a 9:1 mixture of Fmoc-Cl and allyl chloroformate (Alloc-Cl). The resulting resin contained an approximately 5:1 Fmoc:Alloc ratio, as determined by quantifying the amines liberated after Fmoc deprotection versus those liberated after exhaustive removal of both protecting groups. Since each bead has an approximate loading of 300 pmol NH_2, this reserves $\approx$50 pmol as sites for tag addition — more than adequate for the detection limits of their decoding.[15] The resin was treated with piperidine to remove Fmoc, and then acylated with the Fmoc RAM linker. The library was then prepared by first effecting Alloc deprotection with $Pd(PPh_3)_4$/azidotrimethylsilane ($TMSN_3$)/tetrabutylammonium fluoride (TBAF).[15] The resin was portioned into 36 different reaction vessels, and the liberated amines acylated with N-Alloc iminodiacetic anhydride (Scheme 2). The resulting resin-bound carboxylic acids were activated with



Scheme 1. Solid-phase synthesis of *Rauwolfia* alkaloid derivatives. (a) 20% piperidine in DMF; (b) Fmoc-a.a., HATU, DIEA; (c) HATU, DIEA; (d) R_2COOH, 0.5 equiv DIC; (e) DIEA, DMAP; (f) 95/5 TFA/H_2O.

pentafluorophenyl trifluoroacetate, and the active ester displaced with one of 36 mixtures of secondary amine tags, each mixture unique to the individual amino acid that it encodes (Scheme 2).[10] The rates of aminolysis of resin-bound pentafluorophenyl esters are highly dependent on the steric bulk of the secondary amine



Scheme 2. Binary-encoded solid-phase synthesis of a library of acylated rauwolscinamides achieved by cosynthesizing secondary amine tags on a differentially functionalized polymer support.

nucleophiles, and the relative composition of the mixtures of tagging amines were modulated to reflect these kinetic differences.[15] The identity of each amino acid was encoded following a binary tagging strategy[14] using six *N,N*-dialkylamine tags, sufficient to encode up to 63 different building blocks (2^6-1; the 'null' combination is not used).[10] Combinations of three or fewer of the six total tags were sufficient to encode the 36 amino acids used here.[10] Following the introduction of the tags, each reaction vessel was Fmoc-deprotected and then acylated with the corresponding amino acids as described earlier. The resins were pooled, Fmoc-deprotected, and then acylated with rauwolscinic acid (**2b**). The resulting bead mass was portioned into 22 different reaction vessels and then acylated with the carboxylic acids to complete the library. Each resulting bead mass was kept separate, making it unnecessary to encode the carboxylic acids incorporated in the last step.

As a test of the robustness of the encoded library, individual beads were picked at random, cleaved with TFA as described above, and the supernatants analyzed by MS. Each bead was then decoded as described,[15] and the tagging information compared with the mass spectral results. Of the eight beads analyzed in this fashion, seven gave the expected molecular ion while one bead was lost during the decoding process. A representative example of tag analysis from a single bead is shown by the HPLC chromatogram in Figure 2.

Conclusion

The identification of biologically active members from a combinatorial library is often a major challenge since the quantity of material available is frequently insufficient for characterization via conventional chemical analysis. As a result, biologically active pools are often iteratively resynthesized and reassayed as increasingly smaller subsets until one obtains activity data on homogenous compounds.[1] Encoding represents a general solution to this structure elucidation problem. The secondary amines which were utilized as identifier tags serve as a set of surrogate analytes, and once dansylated are detected in sub-picomole amounts. This sensitivity allows the assay of single-beads: a 130 μm TentaGel bead contains approximately 300 pmol of synthesis sites, and if 10% of this is reserved for tagging, the remaining ≈270 pmol are available as sites for ligand synthesis. Liberation of this quantity of ligand into a volume of 100 μL (e.g. in a microtiter well) provides a useful concentration of compound for biological assay (i.e. ≈1 μM, assuming a 50% overall yield for the synthesis). Thus, encoding not only facilitates the identification of active compounds, but also simplifies the biological assay because discrete compounds are tested rather than pools. The natural product libraries described above are presently being screened against a variety of targets ranging from enzymes to single-transmembrane domain receptors, and some of the results of this screening will be reported in due course.

Other approaches to combinatorial natural product diversity include genetically engineered natural product biosynthesis.[16] This technique shows promise as a method for generating combinatorial libraries of specific natural product scaffolds, but is limited by the number of well characterized biosynthetic pathways amenable to this approach — notably polyketides — and by the number of enzymes which can be freely inserted in a given pathway.[17] In another report, Sundram and Griffin described the solid phase derivatization of the important glycopeptide antibacterial agent vancomycin.[18] Several vancomycin–peptide conjugates were synthesized in moderate yield, suggesting that it may eventually be possible to prepare libraries of vancomycin derivatives. As an alternative to the derivatization of a natural product, combinatorial total synthesis could also be considered. However, combinatorial chemistry puts a premium on the number of chemical steps that can be reasonably performed since intermediates are not isolated during the synthesis. A total synthesis approach would require an extremely short, efficient, and general synthetic scheme. As an alternate approach to more comprehensive libraries, the skeleton of a given natural product starting material could be degraded to a synthetically convenient intermediate. This could then serve as a starting material for the combinatorial reassembly of a library of variants of the parent structure. This

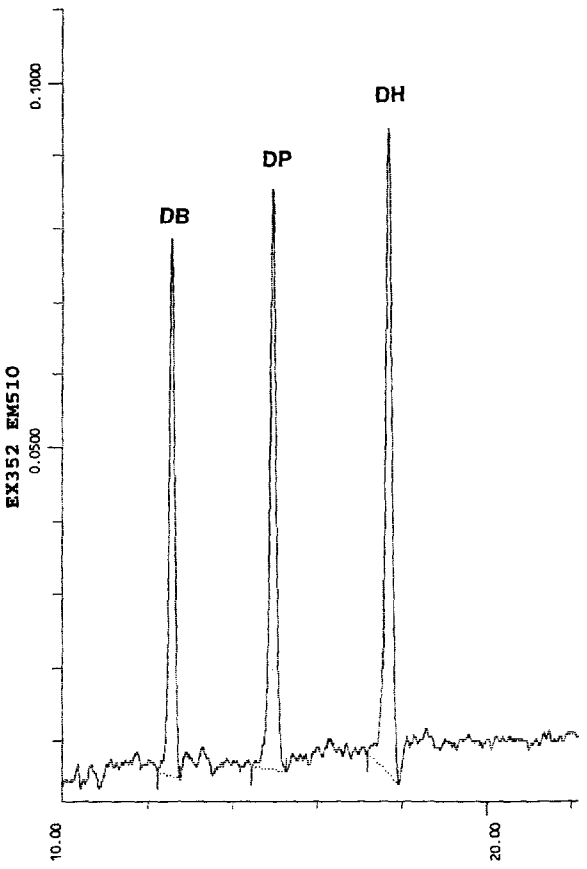


Figure 2. HPLC chromatogram of the dansylated secondary amine tags cleaved from a single bead picked at random from the acylated rauwolscinamide library. The tags DB, DP, and DH reveal that the amino acid incorporated in this bead is phenylalanine (see ref. 10).

approach would undoubtedly require a large investment in methods development.

The approach to combinatorial natural product diversity we report utilizes a natural product as a scaffold for the appendage of building blocks by exploiting the available functional groups on the starting material. A potential limitation of this approach is that it does not permit the combinatorial optimization of the core structure: R_1 and R_2 are easily varied (Scheme 1), but chemistry within the core skeleton is less accessible. This problem is offset by working within a structural family of secondary metabolites. In this way, nature provides related skeletons with different stereochemistry and substituents. If the chosen synthetic scheme is sufficiently general, the same chemistry can be applied to these different starting materials, thus providing a source of diversity within the core structure. A general strategy for the combinatorial modification of natural products which targets existing functionality on the ligands of interest is not only a straightforward method for making large collections of compounds, but is also an historically proven method for creating novel biologically active molecules.[4] It is also a technique which allows the synthesis of libraries of complex chemical structures in a minimum of chemical steps.

Experimental

Yohimbinic acid (Aldrich Milwaukee, WI, U.S.A.), rauwolscine (RBI Natick, MD, U.S.A.), corynanthine (Sigma St. Louis, MO, U.S.A.), reserpine (Aldrich), HATU (PerSeptiva Biosystems, Framingham, MA, U.S.A.), the amino acids (Bachem Bioscience King of Prussia, PA, U.S.A.), and the carboxylic acids (Aldrich) were obtained from the indicated commercial suppliers and used without further purificiation. TentaGel resin was obtained from Rapp Polymere (Tübingon, Germany). Preparative TLC plates (20 × 20 cm × 1 mm; UV254 coating) were obtained from Analtech Network, DE, U.S.A. Additional reagents and solvents were purchased from Aldrich and used without further purification. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Gemini 400 instrument with methanol-d_4 as solvent unless noted. ^{1}H NMR spectral data are reported as follows: chemical shift relative to tetramethylsilane (0.00 ppm), multiplicity, coupling, integration. ^{13}C signals are reported in ppm relative to methanol-d_4 (49.15 ppm). Mass spectra (flow-injection ESI) were obtained on a Finnigan TSQ 7000. High-resolution mass spectra were obtained on a VG zab 2SE.

General procedure for the preparation of acylated yohimbinic aminoacylamides

Method A. To TentaGel-S-NH_2 RAM resin (130 μm diameter, 0.19 mmol NH_2/g, 1.00 g, 0.19 mmol) in a glass reaction vessel containing a fritted glass filter and a stopcock was added a solution of 20% piperidine in DMF (10 mL) and the resin shaken for 15 min. The solvent was drained and a fresh aliquot of piperidine solution added. The resin was shaken an additional 20 min and then washed with DMF and ether. To this was added Fmoc-aminoacid (0.76 mmol; 4 equiv), HATU (0.29 g, 0.76 mmol) and DIEA (0.52 mL, 3.00 mmol) in 7.5 mL DMF and the resin shaken for 2 h. The resin was washed, deprotected with piperidine, and washed again as described above. To this was added yohimbinic acid (0.27 g, 0.76 mmol), HATU (0.29 g, 0.76 mmol) and DIEA (0.52 mL, 3.00 mmol) in 7.5 mL DMF and the resin shaken for 2 h. The resin was washed, dried, and then acylated as follows: to a dry flask under argon was added the desired carboxylic acid (10.5 mmol), anhydrous CH_2Cl_2 (10 mL) and DIC (0.82 mL, 5.25 mmol, 0.5 equiv) and the solution stirred for 30 min. The resulting symmetrical anhydride was added to the resin-bound yohimbinic aminoacyl amide, DIEA (3.66 mL, 21 mmol) and DMAP (75 mg, 0.61 mmol) via syringe through an acrodisc filter and the resin was shaken for 2 h. The acylation procedure was repeated, then the resin suspension was drained, washed, and the alkaloid derivative cleaved from the resin by treatment with 95% TFA/water (8 mL) for 90 min. The filtrate was concd, dissolved in a minimum of 10% MeOH in DCM, and then purified by prep TLC (10% MeOH/DCM). Acylated aminoacylrauwolscinamides were prepared in an analogous manner to rauwolscinic acid.

Method B. Identical to Method A, except the symmetrical anhydride of the carboxylic acid was available commercially and hence not preformed with DIC.

***O*-Cyclohexylpentanoyl-*C*-methioninesulfoneyohimbamide (5).** This derivative was prepared as described above (Method A) from Fmoc-methioninesulfone (0.31 g, 0.76 mmol) and 5-cyclohexylpentanoic acid (1.93 g, 10.5 mmol) to afford 71.3 mg of difunctionalized alkaloid (56%): ^{1}H NMR: δ 7.36 (1H, d, J=2.2 Hz), 7.27 (1H, d, J=2.2 Hz), 7.02 (1H, dd, J=2.2, 1.8 Hz), 6.95 (1H, dd, J=2.2, 1.8 Hz), 5.45 (1H, br s), 4.48 (1H, m), 3.62 (1H, s), 3.36 (1H, d, J=11.7 Hz), 3.1–3.3 (2H, m), 3.02 (3H, s), 2.9 (2H, m), 2.73 (1H, d, J=14.7 Hz), 2.63 (1H, dt, J=4.9, 11.4 Hz), 2.3–2.4 (5H, m), 2.22 (1H, t, J=11.1 Hz), 2.1 (1H, m), 1.98 (1H, q, J=11.2 Hz), 1.9 (1H, m), 1.7 (10H, m), 1.46 (1H, m), 1.35 (1H, m), 1.2 (8H, m), 0.85 (2H, m); ^{13}C NMR: δ 175.9, 175.3, 174.4, 138.5, 136.0, 128.8, 122.4, 120.3, 119.1, 112.7, 108.3, 71.9, 71.7, 62.5, 62.1, 54.5, 53.3, 53.2, 52.4, 50.1, 48.9, 41.4, 41.3, 39.4, 38.8, 38.4, 36.0, 35.1, 34.7, 31.7, 28.3, 28.0, 26.9, 26.7, 25.6, 22.8; MS: $[M+H]^+$ 669. HRMS: calcd for $C_{36}H_{52}N_4O_6S$: 669.3685. Found: 669.3686.

***O*-(8-Bromo)octanoyl-*C*-thienylalanylyohimbamide (6).** This derivative was prepared as described above (Method A) from Fmoc-thienylalanine (0.30 g, 0.76 mmol) and 8-bromoactanoic acid (2.34 g, 10.5 mmol) to afford 65.3 mg of difunctionalized alkaloid (45%): ^{1}H NMR: δ 7.39 (1H, d, J=7.7 Hz), 7.30 (1H, d, J=7.7 Hz), 7.23 (1H, d, J=4.8 Hz), 6.9–7.1 (4H, m), 5.32 (1H, br s), 4.63 (1H, m), 3.44 (2H, t, J=6.6 Hz), 3.28 (2H, m), 3.1 (1H, m), 2.9 (2H, m), 2.73 (1H, d, J=14.7

Hz), 2.63 (1H, dt, $J=4.9$, 11.4 Hz), 2.35 (4H, m), 2.22 (1H, t, $J=11.1$ Hz), 2.0 (2H, m), 1.8 (2H, m), 1.6 (4H, m), 1.4 (10H, m), 1.2 (2H, q, $J=11.8$ Hz); ^{13}C NMR: δ 176.1, 175.0, 174.1, 140.4, 138.5, 136.0, 128.8, 128.4, 128.2, 125.9, 122.5, 120.3, 119.1, 112.7, 108.3, 72.2, 62.5, 62.1, 56.0, 54.5, 53.2, 41.4, 38.6, 35.8, 35.0, 34.7, 34.4, 33.6, 31.3, 30.5, 30.0, 29.5, 26.4, 25.5, 22.8; MS: $[M+H]^+$ 697. HRMS: calcd for $C_{35}H_{45}BrN_4O_4S$: 697.2407. Found: 697.2403.

***O*-Nicotinoyl-*C*-*p*-benzoylphenylalanylyohimbamide (7).** This derivative was prepared as described above (Method A) from Fmoc-*p*-benzoylphenylalanine (0.37 g, 0.76 mmol) and nicotinic acid (1.29 g, 10.5 mmol) to afford 48.8 mg of difunctionalized alkaloid (37%): ^{1}H NMR: δ 9.08 (1H, dd, $J=1.1$, 2.2 Hz), 8.70 (1H, dd, $J=1.83$, 5.13 Hz), 8.32 (1H, m), 7.3–7.8 (11H, m), 6.94–7.04 (2H, m), 5.35 (1H, br s), 4.68 (1H, m), 3.43 (1H, d, $J=11.7$ Hz), 3.3 (1H, m), 3.21 (1H, dd, $J=5.12$, 14.3 Hz), 3.13 (1H, dd, $J=5.5$, 11.0 Hz), 2.92–3.06 (2H, m), 2.64–2.78 (2H, m), 2.53 (1H, dd, $J=2.56$, 11.35 Hz), 2.41 (1H, dt, $J=12.81$, 2.93 Hz), 2.30 (1H, t, $J=11.1$ Hz), 2.1–2.2 (2H, m), 1.78 (1H, m), 1.64 (1H, m), 1.4–1.5 (2H, m), 1.25 (2H, m); ^{13}C NMR: δ 176.3, 173.2, 164.7, 162.8, 138.0, 135.3, 135.1, 134.7, 134.0, 133.9, 133.3, 129.7, 128.8, 128.6, 128.3, 128.2, 127.2, 126.5, 126.4, 124.7, 122.0, 119.8, 118.6, 112.1, 107.7, 72.8, 62.0, 61.6, 55.0, 54.0, 52.5, 40.8, 38.1, 36.9, 36.4, 34.2, 31.0, 25.1, 22.2; MS: $[M+H]^+$ 696. HRMS: calcd for $C_{42}H_{41}N_5O_5$: 696.3185. Found: 696.3188.

***O*-2-Thiophenecarboxy-*C*-1-naphthylalanylyohimbamide (8).** This derivative was prepared as described above (Method A) from Fmoc-1-naphthylalanine (0.33 g, 0.76 mmol) and 2-thiophenecarboxylic acid (1.34 g, 10.5 mmol) to afford 57.4 mg of difunctionalized alkaloid (47%): ^{1}H NMR: δ 8.2 (1H, m), 7.84 (1H, dd, $J=1.1$, 3.7 Hz), 7.78 (1H, m), 7.75 (1H, dd, $J=1.1$, 4.8 Hz), 7.65 (1H, dd, $J=2.7$, 7.0 Hz), 7.3–7.5 (6H, m), 7.16 (1H, dd, $J=4.0$, 4.8 Hz), 6.9–7.0 (2H, m), 5.56 (1H, d, $J=2.9$ Hz), 4.76 (1H, dd, $J=6.4$, 8.1 Hz), 3.58 (1H, dd, $J=6.6$, 13.9 Hz), 3.46 (2H, m), 3.12 (1H, dd, $J=5.7$, 11.3 Hz), 2.94 (2H, m), 2.84 (1H, s), 2.6–2.7 (2H, m), 2.50 (1H, dd, $J=2.5$, 11.7 Hz), 2.2–2.3 (2H, m), 2.1 (2H, m), 1.6–1.8 (4H, m), 1.2 (1H, m); ^{13}C NMR: δ 198.1, 176.1, 173.4, 153.8, 151.2, 143.9, 139.0, 138.9, 137.9, 135.2, 133.5, 131.4, 130.9, 130.1, 129.3, 128.2, 125.0, 121.9, 119.8, 118.5, 112.1, 107.6, 73.5, 71.4, 61.9, 61.6, 55.1, 54.0, 52.4, 40.8, 38.8, 38.2, 34.2, 30.7, 25.0, 22.3; MS: $[M+H]^+$ 647. HRMS: calcd for $C_{38}H_{38}N_4O_4S$: 647.2691. Found: 647.2676.

***O*-(2-Methoxy)cinnamoyl-*C*-cyclohexylalanylyohimbamide (9).** This derivative was prepared as described above (Method A) from Fmoc-cyclohexylalanine (0.30 g, 0.76 mmol) and 2-methoxycinnamic acid (1.87 g, 10.5 mmol) to afford 51.5 mg of difunctionalized alkaloid (42%): ^{1}H NMR: δ 8.0 (1H, d, $J=16.1$ Hz), 7.6 (1H, dd, $J=1.5$, 7.7 Hz), 7.4 (2H, m), 7.3 (1H, d, $J=8.1$ Hz), 7.0 (4H, m), 6.7 (1H, d, $J=16.1$ Hz), 5.5 (1H, d, $J=2.2$ Hz), 4.4 (1H, m), 3.9 (3H, s), 3.5 (1H, d, $J=11.4$ Hz), 3.2 (1H, m), 3.0 (2H, m), 2.7 (4H, m), 2.5 (2H, m), 2.3 (1H, t, $J=11.3$ Hz), 2.1 (3H, m), 1.6 (12H, m), 1.3 (6H, m); ^{13}C NMR: δ 178.4, 173.7, 168.2, 160.0, 142.0, 138.2, 134.8, 133.0, 130.1, 128.2, 124.4, 122.2, 121.9, 120.0, 119.5, 118.7, 112.5, 112.3, 107.6, 72.4, 71.5, 61.9, 56.1, 54.1, 52.2, 52.1, 41.3, 40.7, 37.8, 35.2, 34.2, 33.1, 30.9, 27.6, 27.3, 26.9, 24.9, 23.6, 22.1; MS: $[M+H]^+$ 653. HRMS: calcd for $C_{39}H_{49}N_4O_5$: 653.3703. Found: 653.3687.

***O*-Myristoyl-*C*-serinylyohimbamide (10).** This derivative was prepared as described above (Method B) from Fmoc-Serine(*t*Bu) (0.29 g, 0.76 mmol) and myristic anhydride (4.61 g, 10.5 mmol) to afford 78.5 mg of difunctionalized alkaloid (65%): ^{1}H NMR: δ 7.39 (1H, d, $J=7.7$ Hz), 7.31 (1H, d, $J=8.0$ Hz), 7.03 (1H, ddd, $J=1.1$, 6.95, 8.05 Hz), 6.97 (1H, ddd, $J=1.4$, 7.6, 8.7 Hz). 5.42 (1H, d, $J=2.6$ Hz), 4.39 (1H, dd, $J=5.1$, 4.8 Hz), 3.82 (2H, m), 3.3 (1H, d, $J=10.2$ Hz), 2.95–3.12 (2H, m), 2.90 (1H, dd, $J=3.3$, 11.5 Hz), 2.74 (1H, dd, $J=3.6$, 15.3 Hz), 2.60 (1H, m), 2.4 (5H, m), 2.16 (1H, t, $J=11.0$ Hz), 1.95 (2H, m), 1.6–1.7 (5H, m), 1.55 (2H, m), 1.2–1.5 (20H, m), 0.89 (3H, t, $J=7.0$ Hz); ^{13}C NMR: δ 175.6, 175.3, 174.3, 138.4, 135.8, 128.7, 122.5, 120.3, 119,1, 112.7, 108.2, 72.0, 71.8, 63.6, 62.5, 62.1, 56.8, 54.6, 53.3, 41.3, 38.5, 35.9, 34.6, 33.5, 31.6, 31.2, 31.1, 30.9, 30.7, 26.5, 25.6, 24.2, 22.8, 15.0; MS: $[M+H]^+$ 637.5. HRMS: calcd for $C_{37}H_{57}N_4O_5$: 637.4329. Found: 637.4315.

***O*-Propionyl-*C*-leucinylyohimbamide (11).** This derivative was prepared as described above (Method B) from Fmoc-leucine (0.27 g, 0.76 mmol) and propionic anhydride (1.375, 10.5 mmol) to afford 38 mg of difunctionalized alkaloid (39%): ^{1}H NMR: δ 7.37 (1H, d, $J=7.7$ Hz), 7.28 (1H, d, $J=7.5$ Hz), 7.0 (2H, m), 5.4 (1H, d, $J=2.9$ Hz), 4.3 (1H, dd, $J=4.4$, 10.6 Hz), 3.4 (1H, d, $J=10.3$ Hz), 3.3 (1H, m), 3.15 (1H, dd, $J=5.7$, 11.4 Hz), 2.9–3.0 (2H, m), 2.6–2.8 (2H, m), 2.3–2.4 (3H, m), 2.24 (1H, t, $J=11.1$ Hz), 1.9–2.0 (2H, m), 1.3–1.7 (8H, m), 1.12 (3H, t, $J=7.3$ Hz), 0.96 (3H, d, $J=6.2$ Hz), 0.92 (3H, d, $J=6.2$ Hz); ^{13}C NMR: δ 178.7, 175.5, 174.3, 138.5, 135.8, 128.7, 122.4, 120.2, 118.9, 112.5, 108.1, 72.2, 72.0, 62.5, 62.2, 54.6, 53.3, 52.8, 42.8, 41.4, 38.4, 34.7, 31.5, 29.0, 26.2, 25.4, 24.0, 22.7, 22.2; MS: $[M+H]^+$ 509.2. HRMS: calcd for $C_{29}H_{41}N_4O_4$: 509.3128. Found: 509.3133.

***O*-3-(4-Chlorobenzoyl)propionyl-*C*-cyclohexylalanylrauwolscinamide (12).** This derivative was prepared as described above (Method A) from Fmoc-cyclohexylalanine (0.30 g, 0.76 mmol) and 3-(4-chlorobenzoyl)-propionic acid (2.23 g, 10.5 mmol) to afford 67 mg of difunctionalized alkaloid (51%): ^{1}H NMR: δ 7.97 (2H, d, $J=8.8$ Hz), 7.49 (2H, d, $J=8.8$ Hz), 7.37 (1H, d, $J=7.7$ Hz), 7.3 (1H, d, $J=8.0$ Hz), 7.0 (2H, m), 5.2 (1H, m), 4.5 (1H, m), 3.3 (4H, m), 3.1 (1H, d, $J=11.4$ Hz), 2.9 (4H, m), 2.5–2.8 (6H, m), 2.3 (1H, m), 2.1 (3H, m), 1.9 (2H, m), 1.5–1.8 (6H, m), 0.9–1.4 (6H, m); ^{13}C NMR: δ 199.9, 177.7, 174.3, 173.7, 140.5, 138.1, 136.5, 135.7, 130.8, 130.0, 128.5, 121.9, 120.0, 118.6, 112.1, 108.0, 71.1, 62.5, 61.5, 54.9, 54.0, 52.2, 40.7, 40.1, 37.9, 35.9, 34.9, 34.4, 33.4, 32.0, 29.4, 28.0, 27.5, 25.3,

22.4; MS: $[M+H]^+$ 687.4. HRMS: calcd for $C_{39}H_{48}ClN_4O_5$: 687.3313. Found: 687.3322

***O*-Benzofuranoyl-*C*-serinylrauwoscinamide (13).** This derivative was prepared as described above (Method A) from Fmoc-Serine(*t*Bu) (0.29 g, 0.76 mmol) and benzofurancarboxylic acid (1.70 g, 10.5 mmol) to afford 51.2 mg of difunctionalized alkaloid (47%): ^{1}H NMR: δ 7.74 (1H, d, J=7.7 Hz), 7.56 (2H, m), 7.48 (2H, m), 7.39 (1H, d, J=8.1 Hz), 7.33 (1H, m), 7.19 (1H, m), 7.09 (1H, m), 5.39 (1H, dt, J = 4.4, 11.4 Hz), 4.54 (2H, dd, J=4.8, 6.2 Hz), 3.9 (3H, m), 3.5 (3H, m), 3.3 (1H, m), 3.14 (1H, dd, J=5.1, 11.4 Hz), 3.06 (1H, dd, J=5.1, 16.1 Hz), 2.8 (1H, m), 2.58 (1H, d, J=14.7 Hz), 2.36 (2H, m), 2.0–2.2 (2H, m), 1.8 (1H, m), 1.7 (1H, m); ^{13}C NMR: δ 174.9, 173.3, 160.4, 157.4, 146.8, 138.7, 129.3, 128.6, 127.6, 125.4, 124.6, 124.1, 121.3, 119.6, 115.8, 113.3, 113.1, 107.4, 71.9, 63.6, 59.5, 56.8, 55.3, 53.0, 40.6, 40.4, 40.1, 39.9, 39.7, 38.4, 36.0, 31.8, 26.4, 24.5, 20.4; MS: $[M+H]^+$ 571.3. HRMS: calcd for $C_{32}H_{35}N_4O_6$: 571.2556. Found: 571.2542.

***O*-Undecyleneyl-*C*-leucinylrauwolscinamide (14).** This derivative was prepared as described above (Method A) from Fmoc-leucine (0.27 g, 0.76 mmol) and undecylenic acid (1.93 g, 10.5 mmol) to afford 60.0 mg of difunctionalized alkaloid (51%): ^{1}H NMR: δ 7.4 (1H, d, J=7.3 Hz), 7.34 (1H, d, J=8.4 Hz), 7.0 (2H, m), 5.8 (1H, m), 5.5 (1H, s), 5.2 (1H, m), 4.9 (1H, m), 4.4 (1H, m), 3.4 (1H, d, J=15.0 Hz), 3.1 (1H, d, J=10.6 Hz), 3.0 (2H, m), 2.9 (1H, d, J=11.4 Hz), 2.5–2.8 (4H, m), 2.3 (1H, d, J=11.4 Hz), 2.0–2.2 (5H, m), 1.9 (1H, m), 1.0–1.2 (5H, m), 1.3 (14H, br s), 1.1 (1H, d, J=5.5 Hz), 1.0 (6H, br s); ^{13}C NMR: δ 177.6, 175.0, 174.5, 140.4, 138.2, 136.2, 128.7, 122.3, 120.2, 119.0, 115.2, 112.5, 108.4, 78.3, 70.9, 62.5, 61.8, 55.1, 54.6, 53.2, 42.1, 40.9, 40.1, 38.2, 36.7, 35.6, 35.2, 32.5, 30.7, 30.5, 30.3, 28.3, 26.4, 26.3, 25.6, 23.9, 22.8; MS: $[M+H]^+$ 619.4. HRMS: calcd for $C_{37}H_{55}N_4O_4$: 619.4223. Found: 619.4210.

***O*-(4-Phenyl)butyroyl-*C*-norvalanylrauwolscinamide (15).** This derivative was prepared as described above (Method A) from Fmoc-norvaline (0.26 g, 0.76 mmol) and 4-phenylbutyric acid (1.72 g, 10.5 mmol) to afford 53.4 mg of difunctionalized alkaloid (48%): ^{1}H NMR: δ 7.36 (1H, d, J=7.3 Hz), 7.28 (1H, d, J=8.4 Hz), 7.2 (2H, m), 7.1 (3H, m), 6.9 (2H, m), 5.2 (1H, m), 4.4 (1H, m), 3.11 (1H, d, J=14.6 Hz), 2.95 (2H, m), 2.88 (1H, d, J=14.4 Hz), 2.77 (1H, dd, J=11.8, 6.2 Hz), 2.65 (1H, m), 2.57 (3H, m), 2.50 (1H, m), 2.32 (1H, m), 2.0–2.2 (5H, m), 1.8 (4H, m), 1.7 (2H, m), 1.53 (1H, d, J=14.8 Hz), 1.4 (3H, m), 0.98 (3H, t, J=7.7 Hz); ^{13}C NMR: δ 177.1, 174.5, 174.2, 138.1, 135.6, 129.6, 129.4, 129.3; 128.4, 126.9, 121.9, 119.8, 118.5, 112.1, 108.0, 70.8, 62.4, 61.5, 54.9, 54.3, 54.0, 40.6, 37.9, 36.0, 35.3, 34.6, 32.1, 28.0, 27.9, 25.4, 22.3, 20.3, 14.1; MS: $[M+H]^+$ 585. HRMS: calcd for $C_{35}H_{45}N_4O_4$: 585.3440. Found: 585.3440.

***O*-Acetyl-*C*-tryptophanylrauwolscinamide (16).** This derivative was prepared as described above (Method B) from Fmoc-tryptophan(Boc) (0.40 g, 0.76 mmol) and acetic anhydride (1.07 g, 10.5 mmol) to afford 29.8 mg of difunctionalized alkaloid (28%): ^{1}H NMR: δ 7.73 (1H, d, J=9.8 Hz), 7.35 (1H, d, J=7.6 Hz), 7.27 (1H, d, J=8.0 Hz), 7.20 (1H, s), 7.13 (1H, d, J=8.0 Hz), 6.9–7.1 (4H, m), 5.0 (1H, m), 4.8 (1H, m), 3.6 (1H, s), 3.3 (1H, m), 3.1 (1H, dd, J=15.8, 10.0 Hz), 2.9 (2H, m), 2.75 (1H, d, J=12.8 Hz), 2.65 (2H, m), 2.4 (2H, m), 2.0 (2H, m), 1.9 (1H, m), 1.87 (3H, s), 1.7–1.8 (2H, m), 1.2–1.4 (3H, m); ^{13}C NMR: δ 179.0, 175.4, 173.7, 139.2, 138.9, 136.8, 130.1, 129.6, 126.3, 123.9, 123.2, 121.3, 121.1, 120.8, 119.8, 113.9, 113.4, 112.7, 109.0, 72.6, 72.2, 62.8, 62.4, 56.5, 56.0, 54.8, 42.2, 38.8, 33.0, 30.1, 29.4, 26.4, 23.4, 22.4; MS: $[M+H]^+$ 568. HRMS: calcd for $C_{33}H_{38}N_5O_4$: 568.2923. Found: 568.2924.

***O*-(3-Trifluoromethyl)cinnamoyl-*C*-aspartylrauwolscinamide (17).** This derivative was prepared as described above (Method A) from Fmoc-aspartic acid(tBu) (0.31 g, 0.76 mmol) and 3-trifluoromethylcinnamic acid (2.27 g, 10.5 mmol) to afford 16.2 mg of difunctionalized alkaloid (13%): ^{1}H NMR: δ 7.78 (1H, s), 7.73 (1H, d, J=8.2 Hz), 7.63 (1H, d, J=8.3 Hz), 7.57 (1H, d, J = 8.2 Hz), 7.53 (1H, s), 7.4 (2H, s), 7.0 (2H, m), 6.46 (1H, d, J=17.0 Hz), 5.2 (1H, m), 4.9 (1H, m), 3.92 (1H, d, J=8.0 Hz), 3.42 (1H, d, J=7.9 Hz), 3.26 (1H, d, J=13.2 Hz), 3.1 (2H, m), 2.95 (1H, m), 2.8 (3H, m), 2.6 (2H, m), 2.39 (1H, d, J=14.4 Hz), 2.2 (3H, m), 1.7 (2H, m), 1.5 (1H, m); ^{13}C NMR: δ 178.1, 177.0, 173.5, 167.7, 144.5, 138.7, 137.2, 132.9, 132.8, 131.2, 128.2, 128.0, 127.9, 126.1, 123.0, 121.7, 120.5, 119.1, 112.9, 107.5, 71.3, 63.5, 60.9, 54.9, 52.9, 52.5, 41.4, 39.7, 37.1, 31.9, 27.9, 25.4, 21.4; MS: $[M+H]^+$ 653. HRMS: calcd for $C_{34}H_{36}F_3N_4O_6$: 653.2586. Found: 653.2587.

Preparation of a library of acylated yohimbamides

Thirty-six Fmoc-amino acids[10] were coupled to discrete aliquots of Tentagel-S-NH_2 RAM (0.22 mmol/g, 0.2 g, 0.044 mmol) as described above. The resins were combined, treated with piperidine (20% in DMF; 30 mL), then shaken for 20 min. The resin was washed and then treated with yohimbinic acid (2.27 g, 6.34 mmol), HATU (2.4 g, 6.34 mmol) and DIEA (4.35 mL, 25.0 mmol) in DMF (30 mL). After shaking for 2 h the resin was washed with DMF (2 × 40 mL), ether (2 × 40 mL) and dried. The resin was divided into 22 equal portions, and each portion was treated twice with one of the 22 carboxylic acids[11] as previously described. After washing and drying the resins, the acylated yohimbamides were cleaved from the support by treatment with 95% TFA to give light brown solids.

Preparation of an encoded library of acylated rauwolscinamides

Orthogonally differentiated TentaGel-S-NH_2 resin (5:1, Fmoc:Alloc; 7.5 g, 0.27 mmol/g, 2.0 mmol; prepared as described below) was treated with piperidine (20% in NMP; 30 mL) and shaken for 20 min,

then drained and washed with NMP (3 × 30 mL). Fmoc-RAM (4.32 g, 8.0 mmol) was dissolved in NMP (30 mL) and DIC (0.64 mL, 8.0 mmol) was added. After standing for 5 min the activated linker solution was added to the resin and the mixture shaken for 90 min. Reaction completion was confirmed by negative Kaiser test. The resin was washed with NMP (2 × 30 mL) and DCM (2 × 30 mL). Palladium tetrakis(triphenylphosphine) (0.3 g, 0.4 mmol) was added followed by a mixture of TBAF (1.0 M in THF; 6.0 mL, 6.0 mmol) and $TMSN_3$ (2.1 mL, 16.0 mmol) in DCM (30 mL). The mixture was flushed with nitrogen and shaken for 30 min then drained and washed with DCM (2 × 30 mL) and NMP (30 mL). The resin was treated with N-Alloc-iminodiacetic anhydride (3.2 g, 16 mmol) and DIEA (2.8 mL, 16 mmol) in NMP (30 mL) and shaken for 1 h, then drained and washed with NMP (2 × 30 mL). The resin was treated with pyridine (7.5 mL), NMP (7.5 mL), and pentafluorophenyl trifluoroacetate (7.5 mL) and shaken for 30 min. The resin was drained, washed with NMP (3 × 30 mL), and then split into 36 portions. Each portion was separately treated with an appropriate mixture of secondary amines (ca. 4 mmol) in NMP (1 mL).[10] After shaking for 30 min, the individual resin portions were drained and washed with NMP (3 × 5 mL). Coupling of 36 Fmoc-amino acids was as described above.[10] After pooling the encoded amino acid mixtures, a portion of the resin (2.2 g, 0.6 mmol) was treated with rauwolscinic acid (2.4 mmol, 0.86 g), then split in 22 portions and treated with anhydrides as described above.[11] The final resin samples were not treated with TFA.

Preparation of an orthogonally differentiated (Fmoc/Alloc) acid-cleavable resin

Tentagel-S-NH_2 resin (130 μm diameter, 0.29 mmol NH_2/g, 1.0 g, 0.29 mmol) was treated with a mixture of 9-fluorenylmethyl chloroformate (Fmoc-Cl; 0.54 g, 2.09 mmol) and allyl chloroformate (Alloc-Cl, 24.6 μL, 0.232 mmol) in NMP (10 mL). DIEA (0.4 mL, 2.32 mmol) was added and the suspension shaken at rt for 90 min. The reaction was shown to be complete by negative Kaiser test. Spectrophotometric determination of dibenzofulvene released from a small aliquot of resin upon treatment with piperidine indicated an approximate derivatization ratio of 5:1 Fmoc:Alloc.

Identification of encoded rauwolscinic acid derivatives: correlation between tag and mass spectral results

Eight individual beads were selected at random from the pool of encoded rauwolscinamides acylated with acetic anhydride. Each bead was placed separately in a 200 μL glass vial and TFA (40 μL) was added. After gentle agitation for 40 min, the TFA was removed under vacuum, acetonitrile (40 μL) was added, and the mixture gently swirled. An aliquot was removed for mass spectral analysis. The bead was then retrieved from the vial, placed in a capillary tube, and the tags decoded as previously described.[15]

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References and Notes

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10. Amino acids used in the preparation of libraries, followed in brackets by the binary code of secondary amines used in their encoding (encoding applicable to the acylated rauwolscinamide library only). The numerals one (1) and zero (0) represent the presence or absence, respectively, of a given diamine tag. The following secondary amines were used as molecular tags: *N*-methyl-*N*-heptylamine (MH), *N*-methyl-*N*-dodecylamine (MD), *N*-ethyl-*N*-butylamine (EB), *N,N*-dibutylamine (DB), *N,N*-dipentylamine (DP), and *N,N*-dihexylamine (DH). Tags are listed in the following order: MH, MD, EB, DB, DP, DH. Naturally occurring amino acids are designated by their conventional three-letter abbreviations. Side-chain protecting groups, if present, are indicated in parentheses. All of the amino acids were in their Fmoc-protected forms and L stereoconfigurations. Ala [000001], Asp(tBu) [000010], Gly [000011], Leu [000100], Lys(Boc) [000101], norleucine [000110], Phe [000111], Trp(Boc) [001000], Thr(tBu) [001001], Tyr(tBu) [001010], Val [001011], His(Tos) [001100], Cys(Acm) [001101], γ-glutamic acid(tBu) [001110], His(Bzl) [010000], Arg(Tos) [010001], methionine sulfone [010010], dimethyl phosphotyrosine [010011], Ile [010100], Pro [010101], aminobutyric acid [010110], Glu(tBu) [011000], norvaline [011001], cyclohexyla-

lanine [011010], *p*-benzoylphenylalanine [011100], citrulline [100000], 1-naphthylalanine [100100], methionine sulfoxide [100110], β-alanine [101000], *p*-aminophenylalanine(Boc) [101001], hydroxyproline(tBu) [101010], β-(2-thienyl)alanine [101100], 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid [110000], Ser(OAc) [110010], β-Asp(tBu) [110100], Ser(tBu) [111000].

11. Carboxylic acids employed at R_2: acetic acid, propionic acid, iso-butyric acid, 3-(4-chlorobenzoyl)propionic acid, cyclohexanepentanoic acid, undecylenic acid, 2-methoxyphenylacetic acid, 2-methoxycinnamic acid, 3-(phenylthio)acrylic acid, thioctic acid, 8-bromooctanoic acid, 3-(trifluoromethyl)-cinnamic acid, 11-phenoxyundecanoic acid, 3-(2-methoxyphenyl)propionic acid, 4-ethoxyphenylacetic acid, 3-(3,4-dimethoxyphenyl)propionic acid, 4-phenylbutyric acid, 2-thiophenecarboxylic acid, nicotinic acid, coumalic acid, (*S*)-*O*-acetylmandelic acid, 2-benzofurancarboxylic acid.

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