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The Combined Solid/Solution-Phase Synthesis of Nitrosamines: The Evolution of the "Libraries from Libraries" Concept†

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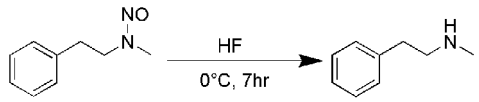
Abstract: The generation of diverse chemical libraries using the "libraries from libraries" concept by combining solid-phase and solution-phase methods is described. The central features of the approaches presented are the use of solid-phase synthesis methods for the generation of a combinatorial polyamine library. Following cleavage from the resin with HF, the polyamine library was reacted with ethyl nitrite in the solution phase to yield the desired nitrosamine library in good yield and purity. The approaches described enable the efficient syntheses of individual nitrosamines as well as mixture-based nitrosamine libraries.

The rapid syntheses of large organic compound collections by combinatorial methods using solid-phase and solution-phase approaches are promising strategies for the discovery of new pharmaceutical lead compounds.[1] These approaches permit the rapid synthesis of large numbers of individual compounds, as well as mixture-based combinatorial libraries, and facilitate their use in high-throughput screening.[2] The focus of this field of research, which initially involved the synthesis of peptides and oligonucleotides, is now on the synthesis of small organic molecules.[3] Recently, a new and unexpected role of nitric oxide (NO) has generated much interest, both as a regulator of many important physiological functions in vivo and as a possible pharmaceutical delivery system.[4] As part of our ongoing efforts directed toward the solid-phase synthesis of small molecules and the generation of combinatorial libraries of organic compounds,[5] we report here an efficient approach for the combined solid/solution-phase synthesis of nitrosamines by expansion of the "libraries from libraries" concept.[6]

Initially, we attempted the synthesis of nitrosamines directly from resin-bound triamines. A variety of conditions were studied to optimize the reaction. However, no nitrosamine product was obtained following cleavage from the resin with HF. It was found that the N−N bond of nitrosamine **1** was cleaved by HF after 7 h at 0 °C to yield the corresponding amine **2** (Scheme 1).To overcome this problem, we modified our "libraries from libraries" concept by first generating the triamines of interest from their corresponding resin-bound dipeptides, and following cleavage, we used solution-phase chemistry to generate the nitrosamines. The synthetic strategy followed is shown in Scheme 2. The parallel solid-phase synthesis of triamines **8** was carried out as described in our previous report[7] using the "tea-bag" methodology.[8] Starting from *p*-methylbenzhydrylamine (MBHA) resin, a Boc-L-amino acid was coupled to the resin. The Boc group was removed using 55% trifluoroacetic acid (TFA) in dichloromethane (DCM). The resulting amine salt was neutralized with DIEA, and the resulting primary amine **4** was then protected with triphenylmethyl chloride (TrtCl). The secondary amide **4** was selectively methylated in the presence of lithium *tert*-butoxide and methyl iodide to yield **5**. Since the alkylation of the amide nitrogen of the resin linkage dramatically increases the acid lability of the MBHA resin-bound peptide, the use of Boc-amino acids was precluded for the second coupling. However, significant amounts of the resin-bound alkylated amino acid are cleaved during the trityl removal, resulting in lower than desired yields based on the loading of the MBHA resin. Therefore, Fmoc-amino acids were employed in the subsequent coupling. Following Fmoc removal and *N*-acylation of the resin-bound dipeptide **6** to afford the resin-bound *N*-acyl dipeptide **7**, exhaustive reduction of the amide bonds using borane in tetrahydrofuran yielded the corresponding resin-bound polyamines **8**. The desired triamine products **9** were obtained following cleavage using HF for 7 h at 0 °C. Since the

SCHEME 1



1 **2**

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† Dedicated to the memory of Professor Henry Rapoport.

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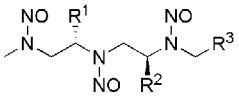
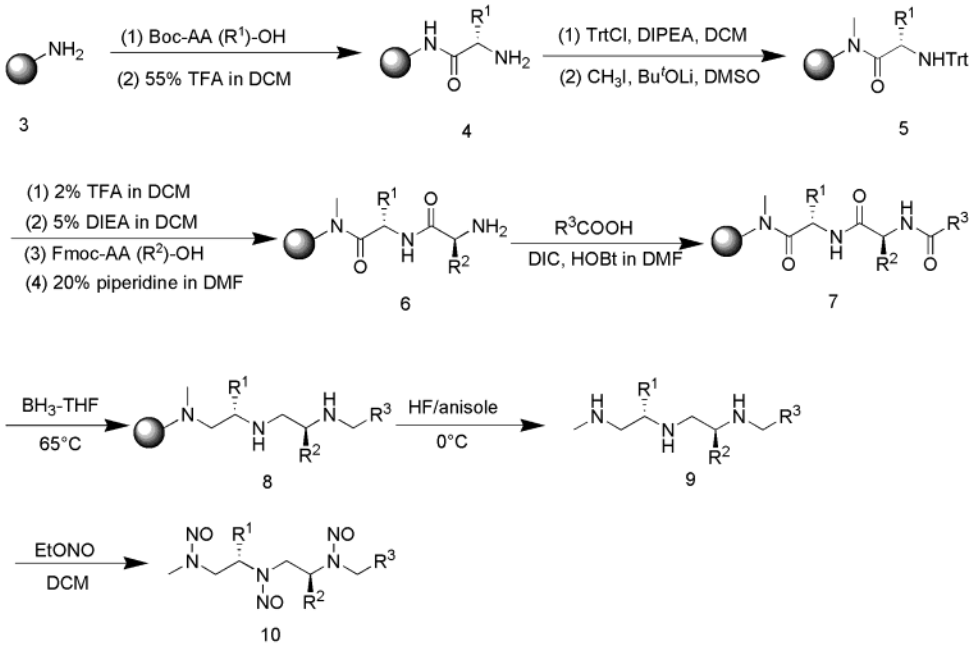
TABLE 1. Individual Nitrosamines 10



entry	product	R^1	R^2	R^3	yield[a] (%)	purity[b] (%)	MW (found)[c]
1	**10a**	CH_3	$C_6H_5CH_2$	$C_6H_5CH_2$	48.2	90	435.4 ([M + Na]$^+$)
2	**10b**	$C_6H_5CH_2$	$C_6H_5CH_2$	$C_6H_5CH_2$	51.3	88	488.6 ([M + Na]$^+$)
3	**10c**	$(CH_3)_2CHCH_2$	$C_6H_5CH_2$	$C_6H_5CH_2$	46.5	94	454.6 ([M + Na]$^+$)
4	**10d**	$(CH_3)_2CH$	$C_6H_5CH_2$	$C_6H_5CH_2$	49.2	92	440.5 ([M + Na]$^+$)
5	**10e**	$C_6H_5CH_2$	$CH_3)_2CHCH_2$	$C_6H_5CH_2$	56.2	89	454.5 ([M + Na]$^+$)
6	**10f**	$C_6H_5CH_2$	$C_6H_5CH_2$	$(CH_2)_5CHCH_2$	52.6	81	494.6 ([M + Na]$^+$)
7	**10g**	$C_6H_5CH_2$	$C_6H_5CH_2$	$(CH_3)_3C$	48.9	83	454.6 ([M + Na]$^+$)
8	**10h**	$C_6H_5CH_2$	$C_6H_5CH_2$	$(CH_3)_2CH$	44.3	82	440.5 ([M + Na]$^+$)

[a] Yields (in %) are based on the weight of crude material and are relative to the initial loading of the resin. [b] The purity of the crude material was estimated based on analytical RP-HPLC traces at $\lambda = 214$ nm. [c] Confirmed by mass spectra (ESI).

SCHEME 2



primary nitrosoamine products are known to be unstable,[9] it was necessary that resin-bound **4** be methylated and that all amines of product **9** be secondary amines

For the next step, the solution-phase synthesis of the nitrosamine library was carried out. We used a large excess of nitroso reagent to drive the reaction to completion and unreacted reagents were simply removed by evaporation. Following optimization of the reaction, it was found that triamine **9** was completely converted to nitrosamine **10** by treatment of **9** with ethyl nitrite (60 equiv) in DCM at room temperature for 5 days. Excess ethyl nitrite was evaporated under vacuum. The products were characterized by electrospray LC-MS under ESI conditions and HRMS. The results are summarized in Table 1.Nitrosamines generally assume planar structures due to the rotational barriers of the N−NO bond and are of similar magnitude to those of amides[10] and carbamates.[11] Unsymmetrical *N*-nitrosamines exist as (*E*)- and

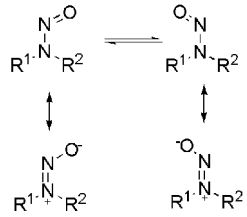


FIGURE 1. The (*E*)-and (*Z*)-rotamers of the unsymmetrical *N*-nitrosamines.

(*Z*)-rotamers due to the stereochemical nature of the N−N═O moiety. Contributing resonance structures give rise to the barrier of rotation[12] (Figure 1).

The ^{1}H NMR spectrum of **1** indicated that there were two rotational isomers in a ratio of 3.1:1 due to the restricted rotation of the N−N bond. There were four rotamers of **10a** seen in the ^{1}H NMR spectrum and it was difficult to specifically attribute the NMR data to

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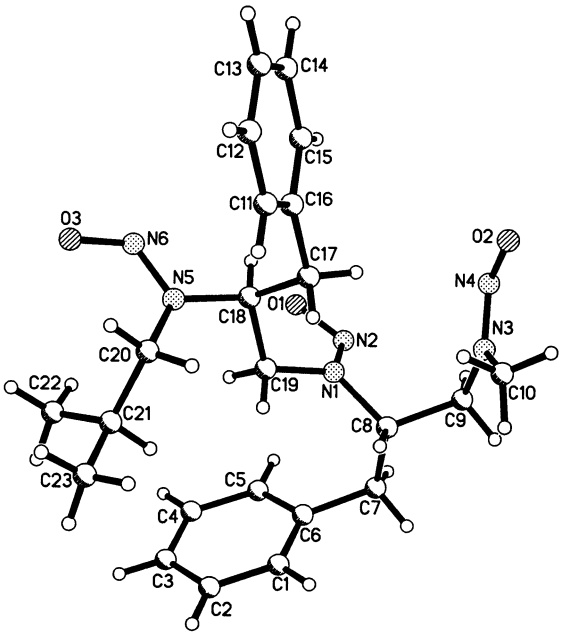

FIGURE 2. X-ray structure of compound **10h**.

given rotamers. A suitable crystal of one of the nitrosamines of **10h** could be grown by slow diffusion of a CH_3CN and CH_3OH solution of **10h** at room temperature. The molecular structure of **10h** is represented in Figure 2. The bond lengths of N2−O1, N4−O2, and N6−O3 are 1.234(2), 1.215(6), and 1.232(3) Å, respectively, which are consistent with their formal double bond. The bond lengths of N1−N2, N3−N4, and N5−N6 are 1.319(3), 1.305(4), and 1.330(3) Å, respectively, closer to a double bond than a single bond. An average distance of the N−N bond in *N*-nitrosamines is 1.31 Å.[13] The bond angles about N3, C10−N3−C9, N4−N3−C9, and N4−N3−C10 are 121.1(3)°, 114.7(3)°, and 124.1(4)°, respectively, are closer to that expected for an sp^2 hybridized nitrogen rather than an sp^3 hybridized nitrogen, suggesting that the pair of electrons formally located on N3 are delocalized as would be expected for *N*-nitrosamines.[12f,13]

Following optimization of the synthetic steps, we expanded the number of individual controls by separately varying the substituent at each of these three variable positions. Ninety-six individual controls were prepared by fixing two positions of diversity while varying the other position. Twenty-nine amino acids were examined at the first position of diversity (R^1), twenty-nine amino acids at the second position of diversity (R^2), and thirty-eight carboxylic acids at the third position of diversity (R^3). The building blocks that produced compounds having purities more than 80% were considered for inclusion in the synthesis of a mixture-based combinatorial library. Amino acids that generated a reactive functionality after reduction (for example, asparagine and glutamine) were not included in the R^1 and R^2 position. We selected nineteen different amino acids at the first position (R^1) of diversity, nineteen different amino acids at the second position (R^2), and thirty-six carboxylic acids at the third position of diversity (R^3) for synthesis of a positional scanning combinatorial library (SCL).[2] The preparation of the mixture-based positional scanning combinatorial library containing 12 996 (19 R^1 × 19 R^2 × 36 R^3) different nitrosamines and its screening in different assays for the identification of highly active compounds will be reported in due course.

In summary, the work presented is a continution of our efforts directed toward the synthesis of acyclic and heterocyclic compounds directly from amino acids and short peptides. Using the concept of "libraries from libraries",we have, thus, been able to generate individual nitrosoamines as well as a mixture-based nitrosamine library from resin-bound *N*-acylated dipeptides.

Experimental Section

Analytical RP-HPLC was performed on a Beckman System Gold Instrument. Samples were analyzed using a Vydac 218TP54 C18 column (0.46 × 25 cm). LC-MS (ESI) was recorded on a Finnigan Mat LCQ mass spectrophotometer at 214 nm using a Betasil C18, 3 μm, 100 Å, 3 × 50 mm column. ^{1}H NMR spectra were recorded at 400 MHz using $CDCl_3$ as solvent. Preparative RP-HPLC was performed on a Waters DeltaPrep preparative HPLC (Millipore) using a Vydac 218TP1022 C18 column (2.2 × 25 cm). X-ray structure determination was performed on a Bruker SMRAT 1KCCD automated diffractometer at The Scripps Research Institute X-ray Facility. Crystals of compound **10h** were obtained by crystallization from methanol and acetonitrile by slow solvent evaporation.

General Procedure for the Solid-Phase Synthesis of Triamines 9.[7] A 100-mg sample of MBHA resin (1 mequiv/g, 100−200 mesh) was contained in a polypropylene mesh packet. Following neutralization with 5% DIEA in DCM, the resin was washed with DCM. The first Boc amino acid (6 equiv, 0.1M) was coupled using hydroxybenzotriazole (HOBt, 6 equiv, 0.1M) and diisopropylcarbodiimide (DICI, 6 equiv, 0.1M) for 90 min. Upon removal of the Boc group with 55% TFA in DCM (30 min), the packet was washed and neutralized with a solution of 5% DIEA in DCM. The resin-bound amine was reacted with trityl chloride in DCM/DMF (9:1) in the presence of DIEA. *N*-Alkylation was performed by treatment of the resin packet with lithium *tert*-butoxide (20 equiv, 1 M) in THF. Excess base was removed by cannulation, followed by addition of CH_3I in DMSO (60 equiv, 0.1 M). The solution was vigorously shaken overnight. Upon removal of the trityl group with 2% TFA in DCM (2 times, 10 min), the packet was washed and neutralized and an Fmoc amino acid was coupled. Following removal of the Fmoc group, the dipeptide was acylated with a carboxylic acid (6 equiv, 0.1 M) in the presence of hydroxybenzotriazole (HOBt, 6 equiv, 0.1 M) and diisopropylcarbodiimide (DICI, 6 equiv, 0.1 M). The exhaustive reduction of the resin-bound amides was carried out in 50-mL glass conical tubes under nitrogen. To each tube was added the resin packet and boric acid (12 equiv). Trimethyl borate (12 equiv) was added followed by the slow addition of borane-THF complex (40 equiv). After cessation of hydrogen evolution, the capped tubes were heated at 65 °C for 72 h in a heating block followed by decantation of the reaction solution and quenching with MeOH. The resin packet was then washed with DMF and MeOH. The resin was treated with piperidine at 65 °C for 20 h to disproportionate the borane complex. Following decantation of the piperidine-borane solution, the resin packet

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was washed with DMF, DCM, and MeOH and dried. Following cleavage of the resin with HF/anisole (95/5) for 7 h at 0 °C, the desired product was extracted with acetic acid/water (95/5) and lyophilized. The product was characterized by electrospray LC-MS under ESI conditions.

General Procedure for the Solution-Phase Synthesis of Nitrosamine 10. A 5.7-mL sample of ethyl nitrite (10 wt % solution in ethyl alcohol, 60 equiv) was added to a solution of triamine **9** (0.1 mmol) in DCM (5 mL) in a 25-mL vial. The reaction mixture was kept at room temperature for 5 days and the solvent was removed via rotary evaporation to yield the corresponding nitrosamine **10**. The product was characterized by electrospray LC-MS under ESI conditions and MALDI-FTMS.

***N*-Methyl-*N*-nitrosophenethylamine 1.** ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.00 (3.57) (s, 3H), 3.05 (2.81) (t, J = 7.2 Hz, 2H), 4.40 (3.80) (t, J = 7.2 Hz, 2H). The NMR spectrum showed that there were two rotational isomers in a ratio of 3.1:1 due to restricted rotation of the N−N bond. The signals of the minor one are shown in parentheses.[14] Compound **1** was treated with HF for 7 h at 0 °C to yield *N*-methylphenethylamine **2**: MS (ESI) m/z 370.1 (M + H$^+$).

1-((1*S*)-1-Benzyl-2-{1-[(1*R*)-1-methyl-2-(1-methyl-2-oxohydrazino)ethyl]-2-oxohydrazino}ethyl)-2-oxo-1-(2-phenylethyl)hydrazine (10a). HRMS (MALDI) m/z calcd for $C_{21}H_{28}N_6O_3Na$ (M + Na)$^+$ 435.2115, found 435.2128.

1-((1*S*)-1-Benzyl-2-{1-[(1*R*)-1-benzyl-2-(1-methyl-2-oxohydrazino)ethyl]-2-oxohydrazino}ethyl)-2-oxo-1-(2-phenylethyl)hydrazine (10b). HRMS (MALDI) m/z calcd for $C_{27}H_{32}N_6O_3Na$ (M + Na)$^+$ 511.2428, found 511.2419.

1-[(1*S*)-1-Benzyl-2-(1-{(1*R*)-3-methyl-1-[(1-methyl-2-oxohydrazino)methyl]butyl}-2-oxohydrazino)ethyl]-2-oxo-1-(2-phenylethyl)hydrazine (10c). HRMS (MALDI) m/z calcd for $C_{24}H_{34}N_6O_3Na$ (M + Na)$^+$ 477.2584, found 477.2578.

1-[(1*S*)-1-Benzyl-2-(1-{(1*R*)-2-methyl-1-[(1-methyl-2-oxohydrazino)methyl]propyl}-2-oxohydrazino)ethyl]-2-oxo-1-(2-phenylethyl)hydrazine (10d). HRMS (MALDI) m/z calcd for $C_{23}H_{32}N_6O_3Na$ (M + Na)$^+$ 463.2428, found 463.2423.

1-[(1*S*)-1-Benzyl-2-(1-{(1*R*)-2-methyl-1-[(1-methyl-2-oxohydrazino)methyl]propyl}-2-oxohydrazino)ethyl]-2-oxo-1-(2-phenylethyl)hydrazine (10e). HRMS (MALDI) m/z calcd for $C_{24}H_{34}N_6O_3Na$ (M + Na)$^+$ 477.2584, found 477.2590.

1-((1*S*)-1-Benzyl-2-{1-[(1*R*)-1-benzyl-2-(1-methyl-2-oxohydrazino)ethyl]-2-oxohydrazino}ethyl)-1-(2-cyclohexylethyl)-2-oxohydrazine (10f). HRMS (MALDI) m/z calcd for $C_{27}H_{38}N_6O_3Na$ (M + Na)$^+$ 517.2897, found 217.2910.

1-((1*S*)-1-Benzyl-2-{1-[(1*R*)-1-benzyl-2-(1-methyl-2-oxohydrazino)ethyl]-2-oxohydrazino}ethyl)-1-neopentyl-2-oxohydrazine (10g). HRMS (MALDI) m/z calcd for $C_{24}H_{34}N_6O_3Na$ (M + Na)$^+$ 477.2584, found 477.2579.

1-((1*S*)-1-Benzyl-2-{1-[(1*R*)-1-benzyl-2-(1-methyl-2-oxohydrazino)ethyl]-2-oxohydrazino}ethyl)-1-isobutyl-2-oxohydrazine (10h). HRMS (MALDI) m/z calcd for $C_{23}H_{32}N_6O_3Na$ (M + Na)$^+$ 463.2428, found 463.2427.

X-ray Single-Crystal Structure Determination of Compound 10h at 296(2) K. Crystal data: $C_{23}H_{32}N_6O_3$, M_r 440.55, monoclinic, space group $P2_1/n$, a = 6.1740(6) Å, b = 20.897(2) Å, c = 9.8368(10) Å, α = 90°, β = 106.255(2)°, γ = 90°, V = 1218.4(2) Å^3, Z = 2, ρ_{calcd} 1.201 Mg/m^3, F_{000} = 472, wavelength (λ) = 0.71073 Å, absorption coefficient (μ) = 0.082 mm^{-1}. **Data collection and reduction**: crystal size = 0.26 × 0.18 × 0.12 mm^3; θ range, 1.95−28.06°; index ranges $-7 \le h \le 8$, $-27 \le k \le 25$, $-12 \le l \le 12$; reflection collected, 9998; independent reflections, 4810 (R_{int} = 0.0319); refinement method, full-matrix least-squares on F^2; date/restraints/parameters, 4810/1/292; final R indices[$I > 2\zeta(I)$]: R1 = 0.0537, wR2 = 0.1304, GOF on F^2 = 1.025, R indices (all data) R1= 0.1018, wR2 = 0.1539, largest difference peak and hole, 0.270 and −0.117 e Å^{-3}.

Acknowledgment. This work was supported by National Cancer Institute Grant No. CA78040 (Houghten).

Supporting Information Available: Copies of LC-MS, HRMS of compounds **10a**−**h**; ^{1}H NMR of compounds **1**, **10a**, and **10b**; tables of crystal data for **10h** (crystal data and structure refinement, atomic coordinates and equivalent isotropic displacement parameters and bond lengths and angles). This material is available free of charge via the Internet at http://pubs.acs.org.

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