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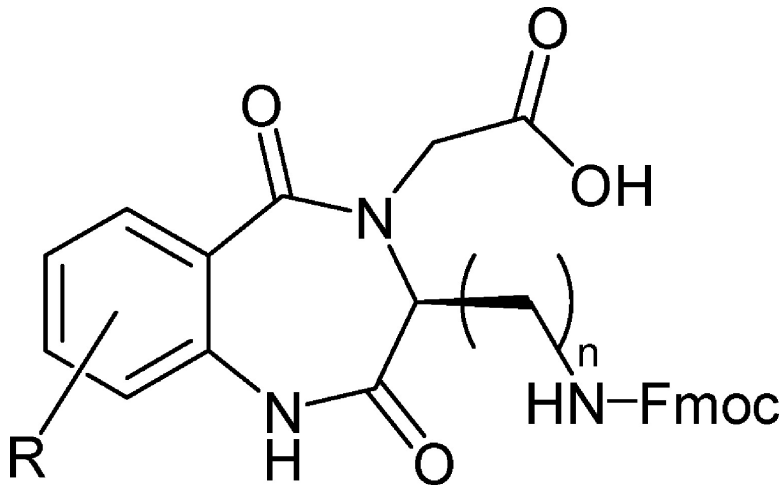

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Solid-Phase Synthesis of 4-Methylcarboxy-1,4-benzodiazepine-2,5-diones

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A solid-phase synthesis of 1,4-benzodiazepinone-2,5-diones is described. This new route can afford benzodiazepinone bearing a *N*-urethane-protected amine and a carboxylic acid function. This kind of building block is valuable as a dipeptide mimic or β-turn mimetic, and it can be introduced in place of any amino acid in peptide synthesis. Using an "analytical probe" strategy, we optimized the synthesis of a model compound on SynPhase Lanterns. Therefore, the efficiency of several linkers was investigated.

Introduction

1,4-Benzodiazepine-2,5-diones are well-known pharmacophores, exhibiting a wide scope of biological activities,[1,2] but they are also described as constrained templates, such as dipeptide mimics.[3,4] Our goal was to develop a new solid-supported route to 1,4-benzodiazepine-2,5-diones as building blocks easily usable in library synthesis or automated SPPS. Numerous synthetic pathways leading to benzodiazepinones have been described in literature. Synthetic strategies belong to three main routes: anchoring the benzodiazepine through the aromatic cycle,[5] cyclization-cleavage from the support,[6−8] and N-anchoring on the diazepine ring.[9,10] However, none lead to building blocks bearing a free carboxylic acid function and a protected amino function suitable for peptide synthesis.

Two different strategies can be set up to insert the benzodiazepine scaffold in a peptide sequence. The first one is the direct preparation of the benzodiazepine moiety during the course of the solid-phase peptide synthesis. As a proof of concept, we synthesized a 32-member pseudopeptide library.[11] Here, we report a second strategy in which the benzodiazepinone scaffold is synthesized on solid support to afford, after cleavage, a *N*-urethane-protected amino acid building block that can be stored and used as standard amino acid in any peptide synthesis.

Results and Discussion

Scheme 1 displays the general solid-phase strategy on Mimotopes SynPhase Lanterns. To quickly optimize the reaction conditions (concentration of reactants, solvent, duration, temperature, etc.) of synthesis steps A−D, an "analytical probe"[12] was anchored on Rink amide PS-Lanterns. Optimization results are presented in Table 1. The analytical probe (HMBA-Phe-Ala−, entry 1, Table 3) enables us to evaluate the conversion percent of each step by a single LC/MS analysis after TFA-mediated cleavage of a slice of lantern.

Step A: Our first attempts with either Mitsunobu activation of HMBA alcohol with DIAD/TPP or coupling of iodoacetic anhydride were unsuccessful. We finally used double-acylation with bromoacetic acid activated by DIC/DMAP to obtain the supported bromo intermediate on HMBA linker (Table 1, entry 5).

Step B: Bromo substitution was successfully performed using paratoluene sulfonate salts of aminoacids benzyl esters neutralized by DIEA. The reaction was complete after 24 h using 0.5 M concentration of amino acid in DMF (Table 1, entries 8−10).

Step C: We first set up several acylation experiments of the supported secondary amine with 2-*N*-fluorenylmethyloxycarbonyl aminobenzoic acid. Indeed, we believed that the use of Fmoc derivative enabled us to use a wide range of acid-labile linkers compatible with mild conditions of Fmoc removal. Thus, we used different coupling reagents including DIC-mediated symmetrical anhydride, HBTU/DIEA, HATU/DIEA, MSNT/NMI, TFFH/DIEA, BTC/pyridine. None of them proved to be effective to acylate the secondary amine (data not shown). We only obtained less than 15% conversion using BTC/pyridine and only when R = H. We also tried to couple unprotected 2-aminobenzoic acid with different coupling reagents including EDC/NMP described by Boo-

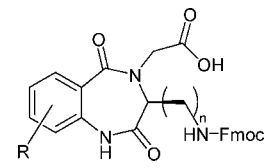

Figure 1. Fmoc-protected 4-methylcarboxy-1,4-benzodiazepine-2,5-dione.

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Scheme 1. Solid-Phase Synthesis of 1,4-Benzodiazepine-2,5-diones

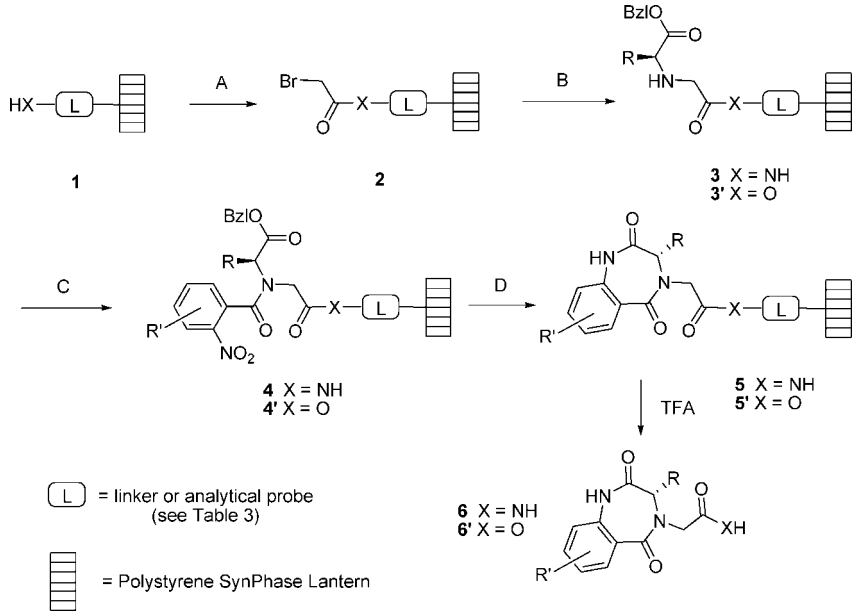


Table 1. Optimization Conditions for Steps A−C on HMBA-Ala-Phe-Rink Amide Linker

step	entry	conditions	R[b]	purity percent of desired compound
A	1	Br-CH_2-COBr (390 mM), TEA (600 mM), 8 h		45%
	2	Br-CH_2-CO_2H (600 mM), DIC (300 mM), 8 h		73%
	3	DIAD (600 mM), triphenylphosphine (600 mM), 30 min then Br-CH_2-CO_2H (600 mM), 8 h		<10%
	4	Br-CH_2-CO_2H (600 mM), DIC (600 mM), DMAP (5 mM), 8 h		77%
	5	Br-CH_2-CO_2H (600 mM), DIC (600 mM), DMAP (5 mM), 8 h, 2 times		>95%
B	6	H-Gly-OBzl 2 M, 24 h, DMSO[a]	H	86%
	7	H-Gly-OBzl, pTsOH 2 M, DIEA 4 M, 24 h	H	83%
	8	H-Gly-OBzl, pTsOH 0.5 M, DIEA 1 M, 24 h	H	89%
	9	H-Leu-OBzl, pTsOH 0.5 M, DIEA 1 M, 24 h	iBu	>95%
	10	H-Ala-OBzl, pTsOH 0.5 M, DIEA 1 M, 24 h	Me	>95%
C	11	2-nitrobenzoylchloride 230 mM, TEA 460 mM, 90 min, rt	H	<50%
	12	2-nitrobenzoylchloride 230 mM, TEA 460 mM, 90 min, 60 °C	H	89%
	13	2-nitrobenzoylchloride 230 mM, TEA 460 mM, 90 min, 60 °C	Me	83%
	14	2-nitrobenzoylchloride 230 mM, TEA 460 mM, 90 min, 60 °C	*i*Bu	88%
	15	2-nitrobenzoic acid 200 mM, DIC 100 mM, 90 min, 2 times, rt	H	71%
	16	2-nitrobenzoic acid 200 mM, DIC 100 mM, 90 min, 2 times, rt	Me	<50%
	17	2-nitrobenzoic acid 200 mM, DIC 100 mM, 90 min, 2 times, rt	*i*Bu	<10%
	18	2-nitrobenzoic acid 200 mM, TFFH 200 mM, DIEA 400 mM, 90 min, 2 times, rt	H, Me, *i*Bu	<50%
	19	2-nitrobenzoic acid 200 mM, HBTU 200 mM, DIEA 400 mM, 90 min, 2 times, rt	H, Me, iBu	<50%
	20	2-nitrobenzoic acid 200 mM, $SOCl_2$ 200 mM, pyridine 700 mM, 90 min, 2 times, rt	H	81%
	21	2-nitrobenzoic acid 200 mM, $SOCl_2$ 200 mM, pyridine 700 mM, 90 min, 2 times, rt	Me	80%
	22	2-nitrobenzoic acid 200 mM, $SOCl_2$ 200 mM, pyridine 700 mM, 90 min, 2 times, rt	*i*Bu	78%
	23	2-nitrobenzoic acid 200 mM, BTC 77 mM, pyridine 760 mM, 90 min, 2 times, rt	H	69%
	24	2-nitrobenzoic acid 200 mM, BTC 77 mM, pyridine 760 mM, 90 min, 2 times, rt	Me	73%
	25	2-nitrobenzoic acid 200 mM, BTC 77 mM, pyridine 760 mM, 90 min, 2 times, rt	*i*Bu	78%

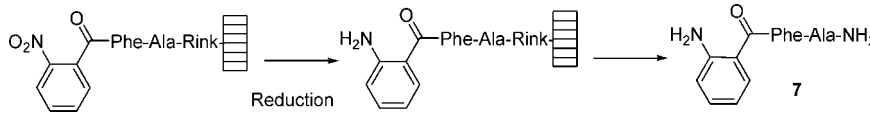
[a] All reactions were performed in DMF except entry 6. [b] The optimization was carried out with no substituents on the aromatic ring (R′ = H).

jamra et al.[10] without any success. We then decided to change slightly our strategy, and we coupled 2-nitrobenzoic acid followed, by a nitro reduction step, to yield free amino group. Acylation of the secondary amine was performed with rather good yield (83−89% purity depending on R group, Table 1, entries 12−14) using 2-nitro benzoic acyl chloride at 60 °C in the presence of triethylamine. Alternatively, $SOCl_2$/pyridine activation of 2-nitro benzoic acid led to better acylation results than other activation agents (∼80% purity; Table 1, entries 20−22).

Step D: Reduction of aromatic nitro group was first performed on a simplified model system (2-nitrobenzoyl-Phe-Ala-Rink amide-PS Lantern). All reaction were carried out in DMF. Results are presented in Table 2. In this simplified model system, sixteen hours at 60 °C treatment with 2 M tin chloride in DMF, followed by a TFA-mediated cleavage step, afforded desired compound **7** with 90% purity.

Using the optimized reduction conditions in entry 7, we moved on to the complete system synthesized on analytical probe linker (Scheme 1, step D, L = HMBA-Ala-Phe-Rink amide). The reduction/cyclization was carried out with three starting supported compounds (R = H, Me, or *i*Bu). After TFA cleavage, no desired benzodiazepine was found, and only the starting analytical probe could be detected by LC/MS analysis: the cylization occurred on the ester bond of the linker and not on the benzylester of the amino acid (Scheme 2). The presence of undesired benzodiazepinone **8a** or **8b** can be detected by LC/MS analysis after DCM extraction of $SnCl_2$ reduction mixture.

Table 2. Optimization of Aromatic Nitro Group Reduction



entry	reducing agent	concentration (M)	reaction time (h)	compound **7** purity (%)
1	NH_4Cl	0.1	0.5	0
	Zn	0.5		
2	$SnCl_2 \cdot 2H_2O$	0.1	0.5	0
3	$SnCl_2 \cdot 2H_2O$	1	0.5	<40%
4	$SnCl_2 \cdot 2H_2O$	2	0.5	<40%
5	$SnCl_2 \cdot 2H_2O$	2	1	75%
6	$SnCl_2 \cdot 2H_2O$	2	3	89%
7	**$SnCl_2 \cdot 2H_2O$**	**2**	**16**	**90%**
8	$SnCl_2 \cdot 2H_2O$	1	0.5	<40%
	NH_4OAc	1		
9	$SnCl_2 \cdot 2H_2O$	1	1	57%
	NH_4OAc	1		
10	$SnCl_2 \cdot 2H_2O$	1	3	61%
	NH_4OAc	1		
11	$SnCl_2 \cdot 2H_2O$	1	21	73%
	NH_4OAc	1		

To validate our synthesis strategy, we repeated experiments with L = Rink amide linker (Table 3, entry 8). With the Rink amide handle, undesired nucleophilic attack cannot occur on amide bond group of the linker, and the cyclization occurred correctly on the benzyl ester of the amino acid affording after TFA cleavage benzodiazepinones **6a** (R = H), **6b** (R = Me), and **6c** (R = *i*Bu) with 87%, 95%, and 71% purity respectively (see Supporting Information for ESI MS and NMR analysis).

However, because our goal was to develop a strategy leading to benzodiazepinone with a free carboxylic function, we started a qualitative study with alcohol linkers. The choice of chemical handle was quite tricky because they should meet two requirements: (a) they should exhibit strong electron-donating effects and/or hindrance to disfavor nucleophilic attack on the "wrong" ester bond, and (b) they should be stable in acidic conditions required to efficient aromatic nitro group reduction. Six other alcohol linkers (Table 3, entries 2−7) were investigated. Only linkers 3, 4, and 5 were commercially available, and we prepared linkers 2, 6, and 7 on Synphase PS Lanterns (Scheme 3; see Supporting Information for detailed synthesis protocols). H-Lys(Fmoc)-OBzl was used as building block in step B. After TFA cleavage of the lantern, the purity percent of desired compound **6′e** was checked by LC/MS analysis. Results are reported in Table 3. MHMPP and trityl linker are not stable during tin chloride 2 M treatment. HMPP and Wang linker are more stable to acidic conditions, but no desired compound **6′e** is detected in LC/MS analysis because of the cyclization on the ester bond of the linker. DHPP linker gave more promising results. Indeed, the expected benzodiapepinone **6′e** was detected (30%) but in the presence of a side product ($[M + H]^+ = 181$) corresponding to breakage of benzylether bond between polystyrene matrix and linker. The same observation has already been described in literature on Rink amide and Wang linkers.[13] Replacement of the benzylether bond by acetamidomethyl linkage is a strategy of choice to avoid this side-reaction. Thus, we prepared the 4-(1,1′-dimethyl-1′-hydroxypropyl) phenoxyacetamidomethyl (DHPPA) linker-functionalized lantern. In this case, **6′e** was obtained with 80% purity. However, we noticed that acylation of hindered tertiary alcohol of DHPPA handle was very difficult and that explained the very low yield obtained (9%). Indeed, despite repetition of coupling step, the presence of alcohol functions was still detected on solid support by colorimetric test performed on a small piece of lantern.[14]

To prove the feasibility of the synthesis, five other compounds (**6′a**−**e** and **6′f**) were prepared on DHPPA linker. Optimized conditions were used with several building blocks. Step B was performed with H-Gly-OBzl, H-Ala-OBzl, H-Leu-OBzl, H-Lys(Fmoc)-OBzl, and H-Dab(Fmoc)-OBzl, and 2-aminobenzoic acid chloride was used as the acylating agent for step C to yield compounds **6′a-e,** whereas compound **6′f** was obtained using 2-amino 5-chloro benzoic acid activated with $SOCl_2$/pyridine. Results are presented in Table 4.

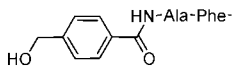
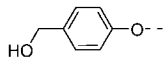
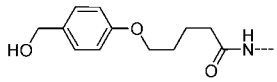
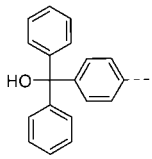
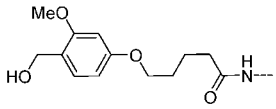
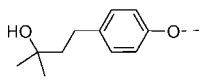
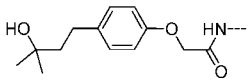
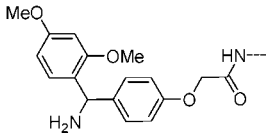
We successfully obtained the targeted benzodiazepinone with low yield but with good purity. Compound **6′b** was resynthesized on five SynPhase DHPPA Lanterns and purified with preparative LC/MS autopurification system to yield enough material to perform ^{1}H NMR and HSQC NMR analysis (Supporting Information).

In this work, we optimized the solid-phase synthesis of 4-methylcarboxy-1,4-benzodiazepine-2,5-diones using SynPhase Lanterns and an analytical probe strategy. To orientate the key step of reduction-cyclization, several linkers were prepared on SynPhase Lanterns and investigated. 4-(1,1′-dimethyl-1′-hydroxypropyl) phenoxyacetamidomethyl (DHPPA) linker enabled us to obtain targeted benzodiazepinones, in particular, some exhibiting a fluorenylmethyloxycarbonyl-protected amino function. These urethane-protected benzodiazepinones can be of great interest as building blocks for peptide synthesis.

Experimental Section

Materials. All the solvents were obtained from Acros and were used without purification. Fmoc-Rink amide (35 μmol), aminomethyl (15 μmol), chloromethyl (15 μmol), trityl alcohol (37 μmol), HMPP (39 μmol), and 3-methoxy HMPP

Table 3. Linkers and Analytical Probe

Entry	HX-L	Full name	Remarks for step D	**6'e** Purity %[a]
1		Hydroxymethylbenzamido (HMBA) –Ala-Phe-Rink amide linker-	Cyclisation occurs on the linker ester bond.	n.a.[b]
2		Wang linker-	8% premature cleavage in $SnCl_2$ reductive conditions[c] Cyclisation occurs on the linker ester bond.	0%
3[b]		Hydroxymethylphenoxypentamido (HMPP) -	40% premature cleavage in $SnCl_2$ reductive conditions[c] Cyclisation occurs on the linker ester bond	0%
4		Hydroxytrityl-	Total premature cleavage in $SnCl_2$ reductive conditions[c]	0%
5		3-methoxy 4-hydroxymethylpentamido- (MHMPP)	Total premature cleavage in $SnCl_2$ reductive conditions[c]	0%
6		4-(1,1'-dimethyl-1'-hydroxypropyl) phenoxyacetamidomethyl- (DHPP)	Side products corresponding to breakage of the ether bond between DHPP linker and PS are observed.	<30%
7		4-(1,1'-dimethyl-1'-hydroxypropyl) phenoxyacetamidomethyl- (DHPPA)		80%
8		Rink amide Linker PS-Lantern		n.a.[b]

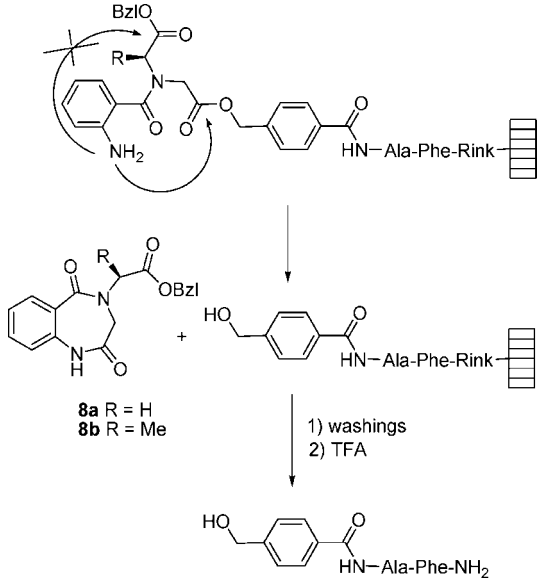
[a] Purity % was determined by LC/MS analysis and integration of the peak area at $\lambda = 214$ nm. [b] Nonapplicable. [c] Premature cleavage was estimated by comparing UV dosage of dibenzofulvene released from Fmoc-Gly-linker-PS lantern before and after $SnCl_2$ 2 M 60 °C 16 h treatment.

(18 μmol) PS SynPhase Lanterns were provided by Mimotopes, Pty, Clayton, Australia. Protected amino acids, TFFH, HBTU, and HATU, were purchased from Senn Chemicals. Other reagents were purchased from Aldrich and Lancaster.

The following abbreviations were used: BTC, *bis* (trichloromethyl carbonate); DCM, dichloromethane; DIC, diisopropylcarbodiimide; DIEA, diisopropylethylamine; DMF, dimethylformamide; TFA, trifluoroacetic acid; HBTU, O-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, N-hydroxybenzotriazole. The other abbreviations used were recommended by the IUPAC-IUB Commission.[15]

LC/MS Analysis. Samples were prepared in acetonitrile/water (50/50 v/v) mixture, containing 0.1% TFA. The LC/MS system consisted of a Waters Alliance 2695 HPLC, coupled to a Micromass (Manchester, U.K.) ZQ spectrometer (electrospray ionization mode, ESI+). All the analyses were carried out using optimized conditions[11] with Chromolith Flash, 25 × 4.6 mm column. A flow rate of 3 mL/min and a gradient of (0−100)% B over 2.5 min were used. Eluent

Scheme 2. Undesired Cleavage on HMBA-Ala-Phe-Rink Analytical Probe



A: water/0.1% HCO_2H. Eluent B: acetonitrile/0.1% HCO_2H. Positive ion electrospray mass spectra were acquired at a solvent flow rate of 200 μL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 100 to 1000 *m/z* in 0.1 s intervals; 10 scans were summed up to get the final spectrum.

LC/MS Purification. Samples were prepared in acetonitrile/water (50/50 v/v) mixture, containing 0.1% TFA. The LC/MS autopurification system consisted of a binary pump Waters 2525, an injector/fraction collector Waters 2676, coupled to a Waters Micromass ZQ spectrometer (electrospray ionization mode, ESI+). All the purifications were carried out using a Waters Symmetry Shield C18 19 × 100 mm, 5-μm particle size, column. A flow rate of 20 mL/min and a gradient of 0−60% B over 20 min were used. Eluent A: water/0.1% TFA. Eluent B: acetonitrile/0.1% TFA. Positive ion electrospray mass spectra were acquired at a solvent flow rate of 204 μL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 100 to 1000 *m/z* in 0.1 s intervals; 10 scans were summed up to get the final spectrum. The collection control trigger is set on single protonated and diprotonated ion with a MIT (minimum intensity threshold) of 8×10^5.

Preparation of the Analytical Probe HMBA-Ala-Phe-Rink Amide Linker-PS Lantern. Standard Fmoc-Deprotection Protocol. The Fmoc-deprotection step was carried out by immersion of the lanterns in a mixture of piperidine and DMF (20:80, v/v) for 60 min. A 200 mL standard flask, equipped with a drilled top was used. After removal of the deprotection solution, the lanterns were washed following the standard washing protocol.

Standard Fmoc SPPS Coupling Protocol. DMF solutions containing Fmoc-amino acid, HBTU, and DIEA were freshly prepared in a standard Schott flask before coupling ([Fmoc-AA-OH] = 120 mM; [HBTU] = 120 mM; [DIEA] = 240 mM). One milliliter of solution per lantern was used (120 μmol, 3.5 equiv of activated amino acid).

The lanterns were immersed for two hours in the coupling solution at room temperature. The solution was decanted, and the lanterns were washed following the standard washing procedure.

Standard Washing Protocol. Washings were performed after each synthetic step, A, B, C, and D. They were carried out by dipping the lanterns in DMF (3 × 5 min), MeOH (2 × 5 min), and DCM (1 × 5 min), respectively. A single 200 mL standard Schott flask, equipped with a drilled top, was used. The lanterns were allowed to air-dry for 15 min after the DCM washing.

Coupling of HMBA linker. DMF solutions containing 4-(hydroxymethyl)benzoic acid, DIC, and HOBt were freshly prepared in a standard Schott flask before coupling ([HMBA] = 200 mM; [DIC] = 200 mM; [HOBt] = 200 mM). One milliliter of solution per Lantern was used (i.e., 200 μmol, 5.7 equiv of activated amino acid).

Scheme 3. Preparation of Wang, DHPP, and DHPPA Linker on SynPhase PS Lanterns

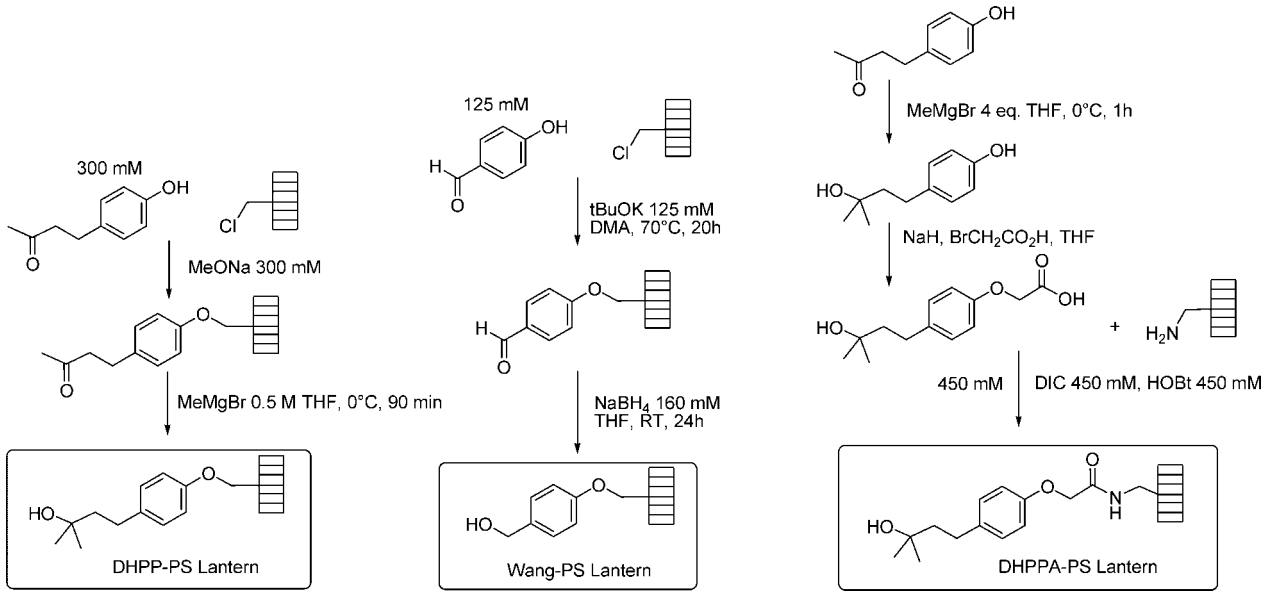


Table 4. Synthesis of 4-Methylcarboxy-1,4-benzodiazepine-2,5-diones on DHPPA-PS SynPhase Lanterns

compound	R	R′	purity (%)[a]	yield (%)
6′a	H	H	99	30
6′b	Me	H	78	9
6′c	*i*Bu	H	95	13
6′d	CH_2-NH-Fmoc	H	72	15
6′e	$(CH_2)_4$-NH-Fmoc	H	80	9
6′f	$(CH_2)_4$-NH-Fmoc	8-Cl	68	13

[a] Purity (%) was determined by LC/MS analysis and integration of the peak area at $\lambda = 214$ nm.

The lanterns were immersed for three hours in the coupling solution at room temperature. The solution was decanted and the lanterns were washed following the standard washing procedure.

Optimized Preparation of Compounds 6 and 6′. Step A: Acylation with Bromoacetic acid. A DMF solution containing bromoacetic acid, DIC, and DMAP was freshly prepared ([Br-CH_2-CO_2H] = 600 mM; [DIC] = 600 mM; [DMAP] = 5 mM). One milliliter of solution per lantern was used (i.e., 300 μmol, 8.5 equiv of symmetrical anhydride (Br-CH_2-$CO)_2O$). The lanterns were immersed for 8 h in the solution at room temperature. The solution was decanted, and the lanterns were immersed again for 8 more hours. Then lanterns were then washed according to the standard washing procedure.

Step B: Substitution of Bromo Derivatives with Amino Acid Benzyl Esters. The lanterns are immersed in a 0.5 M solution DMF solution of aminoacid benzyl ester (H-Leu-OBzl, H-Dap(Fmoc)-OBzl or H-Lys(Fmoc)-OBzl) containing 1 M DIEA for 24 h at room temperature. One milliliter of solution per lantern was used (i.e., 500 μmol, 14.3 equiv of amino acid benzyl ester). The solution was decanted, and the lanterns were submitted to the standard washing protocol.

Step C: Secondary Amine Acylation Protocol. Acylation was done either with acid chloride derivative (a) or with carboxylic acid activated with $SOCl_2$ (b).

(a) Lanterns were treated with a solution of 2-nitrobenzoyl chloride (230 mM) and triethylamine (460 mM) in toluene at 60 °C for 90 min. (i.e., 230 μmol, 6.6 equiv of acyl chloride). The reagent solution was decanted, and the lanterns were washed following the standard washing procedure.

(b) Lanterns were treated with a solution of 2-nitrobenzoic acid or 4-chloro-2-nitrobenzoic acid (200 mM), $SOCl_2$ (200 mM), and pyridine (700 mM) in DMF at room temperature for 90 min (i.e., 200 μmol, 5.6 equiv of amino acid benzyl ester). The reagent solution was decanted, and the lanterns were immersed for 90 more minutes. The lanterns were washed following the standard washing procedure.

Standard Nitro Reduction Protocol. The acylated benzoyl nitro lanterns were treated with a suspension of 2 M $SnCl_2 \cdot H_2O$ in DMF at 60 °C for 16 h. The reagent was decanted. The lanterns were washed following the standard washing procedure.

Cleavage Protocol. A 500 μL aliquot of TFA was dispensed into each polypropylene tube of the deep 96-well plate. Cleavage was carried out for 60 min. The cleavage cocktail was removed from the tubes using a Jouan RC1010 vacuum centrifuge. Compounds were precipitated with dry diethyl ether, centrifuged, and decanted one by one. A 100 μL portion of acetonitrile/water (50/50, v/v) containing a 0.1% TFA was poured into each tube to dissolve the sample. Then the samples were frozen at −80 °C and lyophilized. Precipitation, centrifugation, and decantation were repeated twice to completely remove the volatile residues.

Supporting Information Available. ESI+ LC/MS chromatograms of compounds and ^1H and ^{13}C NMR spectra of resynthesized benzodiazepinones. This material is available free of charge via the Internet at http://pubs.acs.org.

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