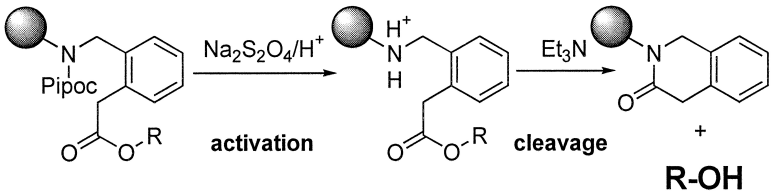
Article

A Cyclitively Cleavable Linker for Alcohols: Linker Preparation and Cleavage Conditions

Xiao, Michael P. Nova, and Anthony W. Czarnik

J. Comb. Chem., **1999**, 1 (5), 379-382• DOI: 10.1021/cc990005w • Publication Date (Web): 18 August 1999



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

A Cyclitively Cleavable Linker for Alcohols: Linker Preparation and Cleavage Conditions

Xiao-Yi Xiao,* Michael P. Nova,† and Anthony W. Czarnik‡

ChemRx, Discovery Partners International, 11149 North Torrey Pines Road, La Jolla, California 92037

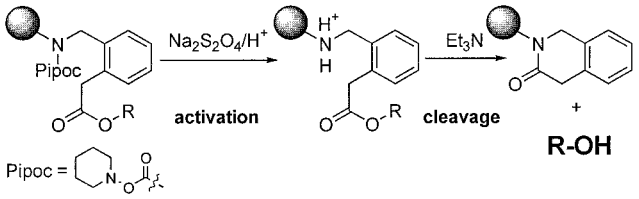
Received February 12, 1999

A new linker has been designed and synthesized using the concept of cyclitive cleavage. The linker is stable in moderately acidic and basic conditions prior to activation. However, once activated under a mild reductive condition, it can be readily cleaved using mild and volatile bases such as triethylamine at room temperature.

A properly designed or chosen chemical linker is crucial not only to enable the use of required chemistries in a solid phase synthetic route but also to facilitate the cleavage of the final product from the solid supports. Most existing linkers are either acid- or base-labile, enabling product cleavage under either acidic or basic conditions.[1] Solid phase syntheses using these linkers are therefore limited to the use of only nonacidic or nonbasic reaction conditions. The sulfonamide-based "safety-catch" linkers[2] are stable to both acids and bases before activation. However, strongly basic or nucleophilic reaction conditions are required in their cleavages, which prevents the final products from bearing any base- and/or nucleophile-labile functionalities such as esters and Michael receptors. In our study of the solid phase synthesis of Taxol analogues,[3] which contain multiple acid-, base-, and nucleophile-sensitive functionalities, we needed a linker that is stable to both moderate acids and bases but can be cleaved under mild, near neutral conditions.

Linkers that cleave as a result of intramolecular cyclization, such as diketopiperazine formation,[4,5] have been used to release both peptides and organic compounds from solid supports. Disadvantageously for our purpose, the activation usually involves deprotection of a Boc-protected precyclizing amine using strong acids (HCl, TFA, etc.), and the subsequent cyclization was generally slow. It has been reported that lactam formation in solution is greatly accelerated when the corresponding amine and acyl groups are preconfigured by incorporating a rigid ring into the precyclic system.[6] We have therefore designed, based on this observation, a new linker that is stable to both moderate acids and bases before activation but can be cleaved cyclitively under mild basic conditions after reductive activation (Scheme 1). These mild cleavage conditions can accommodate the presence of acid-, base-, and nucleophile-sensitive functionalities in the final products. We herein report the preparation and preliminary cleavage study of this new linker (**9**).

Scheme 1



The synthesis of linker **9** is outlined in Scheme 2. Homophthalic anhydride is reduced to diol **1** in 68% yield using LAH in refluxing THF. The diol is then selectively silylated with TBS−Cl to yield allylic alcohol **2** in 64%, which is oxidized by MnO_2 to benzaldehyde **3** in 87%. Reductive amination with a long chain primary amine bearing a MOM ester yielded secondary amine **4** in 46%, which is protected as an *N*-hydroxypiperidinyl carbamate (Pipoc) using Pipoc-OPNP[7] to yield **5** in 48%. Deprotection of the TBS group with TBAF (38% yield), followed by oxidation of the primary alcohol **6** with PDC/DMF, yields carboxylic acid **7**. Carboxylic acid **7** is then protected as the 9-fluorenylmethyl (Fm) ester **(8)**, followed by the removal of the MOM group, yielding the desired new linker **9** (8% for three steps). Yields in the above reactions were not optimized.

Linker **9** was attached onto aminomethyl polystyrene resin (1% DVB) using standard amide chemistry (PyBop/DIEA), affording resin-bound Fm ester **10** (Table 1). To establish the proper activation and cleavage conditions for this linker, the ester resin **10** was subjected to the following procedures in sequence: (1) activation (deprotection of Pipoc with $Na_2S_2O_4$); (2) washing (removal of excess deprotection reagents); and (3) cyclitive cleavage (formation of lactam **11** and release of FmOH). All reactions and procedures were monitored by HPLC analysis of the released FmOH. Uncleaved Fm ester after cleavage was analyzed by Fm ester deprotection with 20% piperidine/DMF followed by measurement of the piperidine adduct of the released 9-methylidenefluorene. As shown in Table 1, activation in HOAc/H_2O left 26% Fm ester uncleaved, probably because the resin did not swell well in HOAc/H_2O and therefore the Pipoc deprotection was too slow (entry 1, Table 1). Indeed, addition of THF to the solvent system decreased the amount of

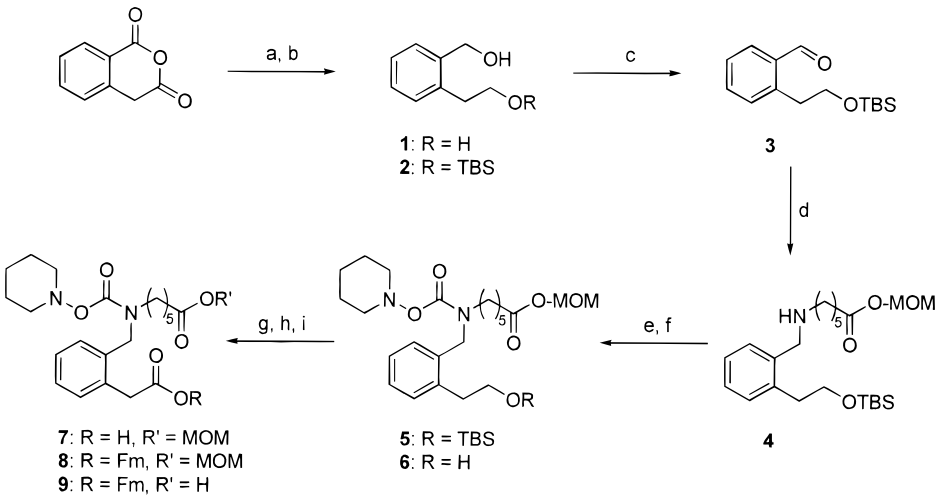
* To whom correspondence should be addressed. Tel: (619) 546-3100. Fax: (619) 546-3083. E-mail: xyxiao@chemrx.com.

† Current address: Graviton Inc., 1043 Juniper St., Carlsbad, CA 92147.

‡ Current address: Illumina, 9390 Towne Centre Drive, STE 200, San Diego, CA 92121-3015.

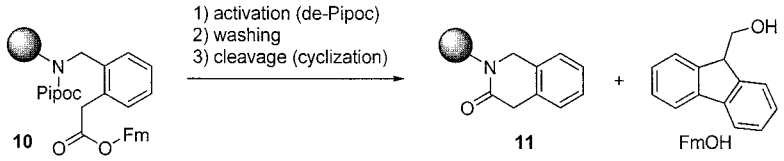
10.1021/cc990005w CCC: $18.00
Published on Web 08/18/1999

Scheme 2[a]



[a] Reagents and conditions: (a) LAH, THF, 75 °C, O.N., 68%; (b) TBS-Cl, Et_3N, 4-DMAP, CH_2Cl_2, rt, O.N., 3 recycling, 64%; (c) MnO_2, hexane, rt, 14 h, 87%; (d) methoxymethyl 6-aminohexanoate, $NaCNBH_3$, HOAc, TMOF, rt, 20 h, 46%; (e) Pipoc-OPNP, DIEA, DMF, rt, O.N., 48%; (f) TBAF, THF, rt, 1 h, 38%; (g) PDC, DMF, rt, 48 h; (h) FmOH, DCC, 4-DMAP, CH_2Cl_2, rt, 10 h, 8% two steps; (i) HCOOH, rt, 6 h, quant.

Table 1. Cleavage of Alcohols from the New Linker



entry	activation conditions	activation time (h)	washing conditions[a]	cleaved FmOH in activation (%)[b]	cleaved FmOH in washing (%)[b]	cleaved FmOH in cyclization (%)[b,c]	uncleaved Fm ester (%)[d]
1	0.25 M $Na_2S_2O_4$ H_2O:HOAc (1:3)	15	10% HOAc/THF	0	0	27	26
2	0.1 M $Na_2S_2O_4$ THF:H_2O:HOAc (5:5:1)	14	10% HOAc/THF	72	<5	22	<5
3	0.1 M $Na_2S_2O_4$ THF:H_2O:HOAc (5:5:1)	1	10% HOAc/THF	14	<5	71	<5
4	0.1 M $Na_2S_2O_4$ THF:H_2O:HOAc (6:6:5)	1	10% HOAc/THF	0	4	62	27
5	0.1 M $Na_2S_2O_4$ THF:H_2O:HOAc (6:6:5)	2	30% HOAc/THF	5	<5	64	<5
6	0.1 M $Na_2S_2O_4$ THF:H_2O:HCOOH (6:6:5)	2	10% HCOOH/THF	0	0	83	<5
7[e]	none	NA[f]	none	0	0	0	100

[a] The resin was washed three times with various solvent systems. [b] The released FmOH was analyzed by HPLC at 254 nm. [c] Cyclization cleavage was performed by treating the resin with 0.5 M Et_3N in benzene at room temperature for 1 h. [d] The uncleaved Fm ester was deprotected by treating the resin with 20% piperidine in DMF at room temperature for 0.5 h. The released 9-methylidenefluorene was measured as the piperidine adduct with a UV spectrometer at 302 nm. [e] Resin was not treated with activation reagents prior to cyclization treatment. [f] NA: not applicable.

uncleaved ester, but most of the ester (72%) cleaved prematurely during the activation step (entry 2, Table 1). A shorter deprotection time (1 h) decreased premature cleavage to 14% (entry 3, Table 1). Greater HOAc concentration virtually eliminated premature cleavage but increased uncleaved ester (entry 4, Table 1). The deprotection time was then increased to 2 h, and both premature cleavage and uncleaved ester decreased to about 5% (entry 5, Table 1). Finally, the use of formic acid as the buffer in place of acetic acid effectively eliminated prematuring cleavage and yielded the liberated FmOH in 83% yield (entry 6, Table 1). One set of effective activation/washing/cleavage conditions for this linker is therefore established as the following: activation by 0.1 M $Na_2S_2O_4$/THF:H_2O:HCOOH (6:6:5, v/v/v) at room temperature for 2 h,[7] washing with 10% HCOOH/THF (three times), and cyclization with 0.5 M Et_3N/benzene at room temperature for 1 h. A control run (no activation, entry 7, Table 1) did not release any alcohol after prolonged treatment (room temperature, up to 24 h) under the same cyclization conditions.

In summary, we have designed a new alcohol linker using the concept of cyclitive cleavage. The linker was synthesized through a reasonable (although not optimized) synthetic route. Cleavage of this new linker can be conducted under very mild conditions via an activation−cyclization protocol. Excess nonvolatile activation reagents are conveniently washed away prior to the release of the final products, greatly facilitating the isolation procedures. In principle, thiols and amines could also be immobilized and cleaved in a similar fashion.[6] Since the *N*-hydroxypiperidinyl carbamate masking group is known to be stable to nucleophiles (amines, thiols, etc.), bases (2 N NaOH, etc.), and acids (TFA, 7 N HCl,

etc.),[7,8] the linker may find broad application in solid phase synthesis of organic compounds, particularly densely functionalized molecules such as natural product analogues. The scope and limitation of this linker strategy are currently under investigation in our laboratories.

Experimental Section

General Procedures. All moisture-sensitive reactions were performed in closed glass vessels under nitrogen. Reagents and solvents were purchased from either Aldrich (Milwaukee, WI) or Fisher (Pittsburgh, PA). Hygroscopic solvents were dried according to standard laboratory procedures prior to use. All reagents and nonhygroscopic solvents were used 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: DIEA, diisopropylethylamine; 4-DMAP, 4-(dimethylamino)pyridine; LAH, lithium aluminum hydride; TBAF, tetrabutylammonium fluoride; PDC, pyridinium dichromate; DCC, dicyclohexyl carbodiimide; TFA, trifluoroacetic acid; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrafuran; TMOF, trimethylorthoformate; Boc, *t*-butoxycarbonyl; TBS, *t*-butyldimethylsilyl; MOM, methoxymethyl; Pipoc, *N*-piperidinooxycarbonyl; PNP, *p*-nitrophenyl; FmOH, 9-fluorenemethanol.

2-Hydroxymethylphenethyl Alcohol (1). Homophthalic anhydride (24.80 g, 85% purity, 130.0 mmol) was dissolved in dry THF (200 mL). LAH (1.0 M in THF, 184 mL, 184 mmol, 1.4 equiv) was added dropwise at room temperature. The resulting brown solution was stirred at room temperature for 1 h and at 75 °C overnight. The reaction was cooled to room temperature with a cold water bath and carefully quenched with methanol (25 mL). The quenched reaction solution was then slowly poured into a vigorously stirred 10% NaOH aqueous solution (500 mL) and stirred for 30 min (until the solution became clear). The mixture was extracted with diethyl ether (200 mL × 3). The extracts were combined, dried over magnesium sulfate, concentrated, and flash column chromatographed on silica gel with 40−80% EtOAc/petroleum ether to yield a pale oil **1** (13.43 g, 68%): $R_f = 0.55$, EtOAc; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (d, $J = 7.6$ Hz, 1 H), 7.30 (d, $J = 6.6$ Hz, 1 H), 7.20−7.25 (m, 2 H), 4.68 (s, 2 H), 3.93 (t, $J = 5.7$ Hz, 2 H), 2.98 (t, $J = 5.7$ Hz, 2 H).

2-Hydroxymethylphenethyl *t*-Butyldimethylsilyl Ether (2). Diol **1** (13.00 g, 85.42 mmol) was dissolved in dry CH_2Cl_2 (50 mL). Et_3N (14.29 mL, 102.4 mmol, 1.2 equiv) and 4-DMAP (1.04 g, 8.51 mmol, 0.1 equiv) were added. A solution of TBS-Cl (12.88 g, 85.45 mmol, 1.0 equiv) in CH_2Cl_2 (170 mL) was slowly added to the reaction mixture with constant stirring at room temperature over an 8 h period. The reaction mixture was further stirred overnight at room temperature, diluted with ether (400 mL), washed with saturated sodium bicarbonate (100 mL × 3) and brine (100 mL × 1), dried over magnesium sulfate, concentrated, and flash chromatographed on silica gel with 5−15% EtOAc/petroleum ether to yield 6.34 g (28%) of the desired silyl ether **2** and its regioisomer and starting material, which was recycled. A yield of 14.40 g (64%) of silyl ether **2** was obtained after three rounds of recycling: $R_f = 0.60$, 10% EtOAc/petroleum ether; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19−7.35 (m, 4 H), 4.64 (s, 2 H), 3.90 (t, $J = 5.8$ Hz, 2 H), 2.95 (t, $J = 5.8$ Hz, 2 H), 0.79 (s, 9 H), −0.06 (s, 6 H).

2-Formylphenethyl *t*-Butyldimethylsilyl Ether (3). Benzyl alcohol **2** (14.40 g, 54.04 mmol) was dissolved in hexane and treated with activated MnO_2 (55.30 g, 85% purity, 541 mmol, 10 equiv) at room temperature for 14 h. The solid was filtered off, and the colorless solution was concentrated and rapidly chromatographed on silica gel with 0−5% EtOAc/hexane to yield 12.37 g (87%) of the desired aldehyde **3** as a colorless oil: $R_f = 0.55$, 40% EtOAc/petroleum ether; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.29 (s, 1 H), 7.82−7.86 (m, 1 H), 7.25−7.54 (m, 3 H), 3.85 (t, $J = 6.6$ Hz, 2 H), 3.24 (t, $J = 6.6$ Hz, 2 H), 0.82 (s, 9 H), −0.06 (s, 6 H).

Methoxymethyl *N*-(2-*t*-Butyldimethylsiloxyethylbenzyl)-6-aminohexanoate (4). Benzyl aldehyde **3** (1.25 g, 4.73 mmol) and methoxymethyl 6-aminohexanoate (1.00 g, 5.68 mmol, 1.2 equiv) were dissolved in anhydrous TMOF (20 mL) and stirred at room temperature for 20 h. Sodium cyanoborohydride (0.60 g, 9.55 mmol, 2.0 equiv) was dissolved in anhydrous TMOF (10 mL) and added dropwise into the reaction mixture at room temperature. After the mixture was stirred for 10 min, acetic acid (0.30 mL, 1% v/v) was added dropwise, and the reaction mixture was stirred at room temperature for 7 h. The reaction solution was diluted with EtOAc (200 mL), washed with saturated sodium bicarbonate (50 mL × 3) and brine (50 mL × 1), dried over magnesium sulfate, concentrated, and chromatographed on silica gel with 1−5% MeOH/CH_2Cl_2 to yield 0.91 g (46%) of the desired product **4** as an oil: $R_f = 0.33$, 5% MeOH/CH_2Cl_2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32−7.10 (m, 4 H), 5.22 (s, 2 H), 3.85 (s, 2 H), 3.82 (t, $J = 7.0$ Hz, 2 H), 3.46 (s, 3 H), 2.89 (t, $J = 7.0$ Hz, 2 H), 2.72 (t, $J = 7.4$ Hz, 2 H), 2.37 (t, $J = 3.7$ Hz, 2 H), 1.79−1.30 (m, 6 H), 0.84 (s, 9 H), −0.03 (s, 6 H); LRMS (electrospray) *m*/*z* $[M + H]^+$: 424, calcd for $C_{23}H_{41}NO_4Si$: 423.

Methoxymethyl *N*-(2-*t*-Butyldimethylsiloxyethylbenzyl)-*N*-Pipoc-6-aminohexanoate (5). The above secondary amine **4** (0.90 g, 2.12 mmol) was dissolved in anhydrous DMF (20 mL). DIEA (0.56 mL, 3.20 mmol, 1.5 equiv) and Pipoc-OPNP (0.79 g, 3.00 mmol, 1.4 equiv) were then added sequentially. The reaction mixture was stirred at room temperature overnight, diluted with diethyl ether (200 mL), washed with saturated sodium bicarbonate (50 mL × 3) and brine (50 mL × 1), dried over magnesium sulfate, concentrated, and chromatographed on silica gel with 20−40% EtOAc/petroleum ether to yield the desired product as an oil (0.56 g, 48%): $R_f = 0.25$, 40% EtOAc/petroleum ether; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30−7.10 (m, 4 H), 5.22 (s, 2 H), 4.51 (br s, 2 H), 3.77 (t, $J = 6.8$ Hz, 2 H), 3.46 (s, 3 H), 3.40 (br s, 2 H), 3.18 (br s, 2 H), 2.84 (t, $J = 6.8$ Hz, 2 H), 2.60 (br s, 2 H), 2.32 (t, $J = 7.5$ Hz, 2 H), 1.85−1.50 (m, 12 H), 0.86 (s, 9 H), −0.03 (s, 6 H); LRMS (electrospray) *m*/*z* $[M + H]^+$: 551, calcd for $C_{29}H_{50}N_2O_6Si$: 550.

Methoxymethyl *N*-(2-Hydroxyethylbenzyl)-*N*-Pipoc-6-aminohexanoate (6). TBAF (2.0 mL, 1.0 M in THF, 2.00 mmol, 2.0 equiv) was added to a THF (5 mL) solution of the TBS ether **5** (0.56 g, 1.02 mmol) at room temperature.

After being stirred at room temperature for 1 h, the reaction mixture was diluted with water (100 mL) and extracted with diethyl ether (50 mL × 3). The extracts were combined, dried over magnesium sulfate, concentrated, and chromatographed on silica gel using 50−100% EtOAc/petroleum ether to yield alcohol **6** as an oil (0.17 g, 38%): $R_f = 0.40$, EtOAc; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.13 (m, 4 H), 5.22 (s, 2 H), 4.55 (br s, 2 H), 3.81 (br t, $J = 6.4$ Hz, 2 H), 3.46 (s, 3 H), 3.40 (br s, 2 H), 3.16 (br s, 2 H), 2.91 (t, $J = 6.4$ Hz, 2 H), 2.62 (br s, 2 H), 2.33 (t, $J = 7.5$ Hz, 2 H), 1.82−1.20 (m, 12 H); LRMS (electrospray) m/z $[M + H]^+$: 437, calcd for $C_{23}H_{36}N_2O_6$: 436.

Methoxymethyl *N*-[2-(9-Fluorenylmethoxycarbonylmethyl)benzyl]-*N*-Pipoc-6-aminohexanoate (8). Phenethyl alcohol **6** (0.17 g, 0.39 mmol) was treated with PDC (0.88 g, 2.34 mmol, 6.0 equiv) in DMF (5 mL) at room temperature for 48 h. The solution was diluted with saturated NaH_2PO_4 (pH 3−4, 100 mL) and extracted with EtOAc (50 mL × 4). The extracts were combined, dried over magnesium sulfate, and concentrated under vacuum. The dried crude product was then dissolved in dry CH_2Cl_2 (2 mL) and treated with excess of FmOH, 4-DMAP, and DCC (5 equiv each) at room temperature for 10 h. The solution was concentrated, and the residue was chromatographed on a preparative silica gel TLC with 50% EtOAc/petroleum ether to yield 18 mg (8% overall) of the desired Fm ester **8** as an oil: $R_f = 0.75$, EtOAc; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85−7.25 (m, 12 H), 5.20 (s, 2 H), 4.52 (br s, 2 H), 4.36 (br d, $J = 6.9$ Hz, 2 H), 4.15 (t, $J = 6.9$ Hz, 1 H), 3.76 (br s, 2 H), 3.44 (s, 3 H), 3.50−2.50 (m br s, 6 H), 2.28 (t, $J = 7.5$ Hz, 2 H), 2.00−1.20 (m, 12 H); LRMS (electrospray) m/z $[M + H]^+$: 629, calcd for $C_{37}H_{44}N_2O_7$: 628.

***N*-[2-(9-Fluorenylmethoxycarbonylmethyl)benzyl]-*N*-Pipoc-6-aminohexanoic Acid (9).** MOM ester **8** (18 mg, 0.029 mmol, 1 equiv) was treated with formic acid at room temperature for 6 h. The solution was concentrated under vacuum to yield 17 mg (quantitative) of the desired carboxylic acid **9**: $R_f = 0.40$, 10% MeOH/CH_2Cl_2; LRMS (electrospray) m/z $[M + H]^+$: 585, calcd for $C_{35}H_{40}N_2O_6$: 584.

***N*-[2-(9-Fluorenylmethoxycarbonylmethyl)benzyl]-*N*-Pipoc-6-aminohexanoyl-NH-polystyrene Resin (10).** Linker **9** (17 mg, 0.029 mmol, 1.0 equiv) was reacted with excess aminomethyl polystyrene resin (0.40 g, 0.45 mmol/g, 0.18 mmol, 6.0 equiv), DIEA (0.02 mL, 0.12 mmol, 4.0 equiv), and PyBop (30 mg, 0.058 mmol, 2.0 equiv) in dry CH_2Cl_2 (4 mL) at room temperature for 20 h. TLC analysis of the supernatant indicated that all the linker had been consumed. The resin was washed with THF and diethyl ether alternately (10 mL × 4) and dried under vacuum. The excess amino groups on the resin were then capped with Ac_2O/DIEA (0.5 M each, 4 mL) at room temperature for 0.5 h. The Kaiser test[10] was negative, indicating the absence of free amino groups. The capped resin was washed and dried as above to yield resin **10**.

General Activation Procedure for the New Linker. Resin **10** was treated with $Na_2S_2O_4$ in THF/H_2O/HCOOH (or HOAc) at room temperature for 1−15 h, washed with HCOOH (or HOAc)/THF three times, and dried under vacuum. See Table 1 and main text for details.

General Cleavage Procedure for the New Linker. The above activated resin was treated with 0.5 M Et_3N/benzene at room temperature for 1 h. The resin was filtered off, and the filtrate was analyzed for the released FmOH with HPLC. See main text and Table 1 for details.

Acknowledgment. We thank Professors K. C. Nicolaou (The Scripps Research Institute) and Emmanuel Theodorakis (University of California−San Diego), and ChemRx/IRORI colleagues Drs. Shuhao Shi and Rognshi Li for assistance and valuable discussions. Professor K. C. Nicolaou is an advisor to ChemRx/IRORI.

References and Notes

(1) *A Practical Guide to Combinatorial Chemistry*; DeWitt, S. H., Czarnik, A. W., Eds.; American Chemical Society: Washington, DC, 1997.

(2) Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. The Safety Catch Principle in Solid Phase Peptide Synthesis. *J. Chem. Soc., Commun.* **1971**, 636−637. Another safety-catch type linker is based on the bis-*p*-methylsulfinyl benzhydrylamine, which utilizes Me_3SiBr/TFA/thioanisole for cleavage: Pátek, M.; Lebl, M. Safety-catch Anchoring Linkage for Synthesis of Peptide Amides by Boc/Fmoc Strategy. *Tetrahedron Lett.* **1991**, *32*, 3891−3894.

(3) Xiao, X.-Y.; Prandoosh, Z.; Nova, M. P. Design and Synthesis of a Taxoid Library Using Radio Frequency Encoded Combinatorial Chemistry. *J. Org. Chem.* **1997**, *62*, 6029−6033.

(4) Maeji, N. J.; Bray, A. M.; Geysen, H. M. Multi-pin Peptide Synthesis Strategy for T Cell Determinant Analysis. *J. Immunol. Methods* **1990**, *134*, 23−33.

(5) Burgess, K.; Jacutin, S. E.; Lim, D.; Shitangkoon, A. An Approach to Photolabile, Fluorescent Protecting Groups. *J. Org. Chem.* **1997**, *62*, 5165−5168.

(6) Entwistle, I. D. The Use of 2-Nitrophenylpropionic Acid as a Protecting Group for Amino and Hydroxy Functions to be Recovered by Hydrogen Transfer Reduction. *Tetrahedron Lett.* **1979**, 555−558.

(7) Stevenson, D.; Young, G. T. Amino acids and Peptides. Part XXXI. *N*-(Piperidino-oxycarbonyl)amino acids and their Use in Peptide Synthesis. *J. Chem. Soc.* **1969**, *C*, 2389−2398.

(8) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; John Wiley & Sons: New York, 1991; Chapter 7, pp 334, 441−444 and references therein.

(9) Troll, W.; Cannan, R. K. A Modified Photometric Ninhydrin Method for the Analysis of Amino and Imino Acids. *J. Chem. Soc.* **1952**, *155*, 803−811.

CC990005W