JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Triazene as a Powerful Tool for Solid-Phase Derivatization of Phenylalanine Containing Peptides: Zygosporamide Analogues as a Proof of Concept

Carolina Torres-García,[†,‡] Daniel Pulido,[‡,§] Fernando Albericio,[#,†,∥,§,⊥] Miriam Royo,*[,‡,§] and Ernesto Nicolás*[,†]

[†]Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain

[‡]Unitat de Química Combinatòria, Parc Científic de Barcelona, 08028 Barcelona, Spain

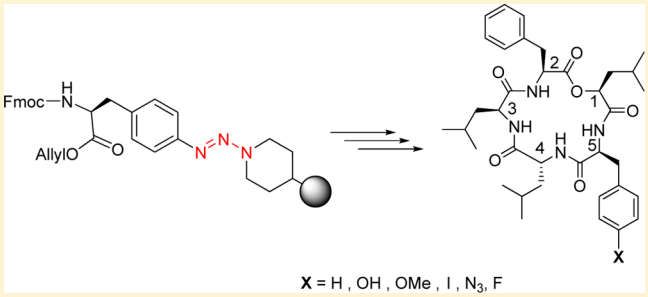
[§]Biomedical Research Networking in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 08028 Barcelona, Spain

[∥]Institute for Research in Biomedicine (IRB Barcelona), 08028 Barcelona, Spain

[⊥]School of Chemistry, University of KwaZulu-Natal, 4001 Durban, South Africa

Supporting Information

ABSTRACT: A novel method for the synthesis of *para*-substituted phenylalanine containing cyclic peptides is described. The main features of this strategy are the coupling of phenylalanine to the solid support through its side chain via a triazene linkage, on-resin cyclization of the peptide chain, cleavage of the cyclic peptide from the resin under mild acidic conditions and further transformation of the resulting diazonium salt. The usefulness of this approach is exemplified by the solid-phase synthesis of some derivatives of the naturally occurring cyclic depsipeptide zygosporamide.



INTRODUCTION

Naturally occurring cyclic peptides have become relevant tools as scaffolds for the development of peptide-based drugs.[1] Features that contribute to this fact are the conformational rigidity of the ring that allows the enhanced binding toward target molecules, resistance to degradation by exopeptidases, higher lipophilicity and membrane permeability than their linear counterparts. These properties confer them a key role as potential candidates for the design of new compounds with improved biological properties.

Among the amino acids that are usually found in natural peptides, phenylalanine (Phe) is of particular interest from the molecular recognition point of view. The aromatic side chain of this amino acid is responsible for a number of hydrophobic intermolecular interactions with receptors or intramolecular interactions for structural stabilization that can be crucial for intrinsic activity.[2] Furthermore, Phe and its derivatives with modifications in its aromatic ring have become key pharmacophores in SAR studies of many biologically active peptides and peptidomimetics.[3] For this reason, there is a need for strategies to efficiently synthesize peptides containing this amino acid with diverse derivatizations on its aromatic ring. In particular, strategies that take advantage of the solid-phase methodology are desirable in order to implement combinatorial approaches. In this sense, anchoring of Phe through its side chain to a polymeric support via triazene[4] provides a site for eventual derivatization of this amino acid after cleavage. Thus, the peptide cleavage from the resin affords an aromatic diazonium salt that can be easily modified. Tethering an aromatic ring to a polymeric support through a triazene linker was pioneered by Bräse et al.[5] for the solid-phase synthesis of aromatic and heteroaromatic compounds.[6] This approach is based on coupling an aromatic amine through its diazonium salt to a secondary amino-functionalized polymeric support. The robust triazene linker is compatible with a wide range of reaction conditions. However, acidic cleavage of the triazene resin under mild conditions yields the amine resin and the modified aryldiazonium salt that can be further reduced to afford the target molecule in a traceless manner[5,7] or chemically transformed to introduce diverse functionalities at the aromatic ring.[8] We recently demonstrated the feasibility of this approach as a traceless methodology for the synthesis of *C*-terminal derivatized peptides and cyclic peptides.[9] With the aim of expanding the scope of application of triazene linker in the peptide chemistry field, we decided to explore its use for the introduction of chemical diversity in peptides.

RESULTS AND DISCUSSION

Herein we report our results on the use of this strategy in the solid-phase synthesis of peptide analogues based on different Phe side chain modifications at the *para* position of the ring.

Received: August 7, 2014
Published: November 10, 2014

dx.doi.org/10.1021/jo501830w | J. Org. Chem. 2014, 79, 11409−11415

Zygosporamide, a potent cytotoxic natural cyclic depsipeptide, isolated from the marine-derived fungus *Zygosporium masonii*[10] has been used as model peptide (**1a**, Figure 1). This

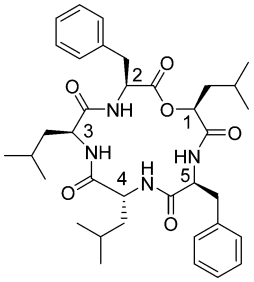


Figure 1. Structure of zygosporamide (**1a**).

depsipeptide contains five residues: two leucines, two phenylalanines and a α-hydroxyisocaproic acid. Ma and co-workers recently reported a solution synthesis of zygosporamide and a series of four Ala-substituted zygosporamide analogues. These compounds displayed selective cytotoxicity against a variety of cancer cell lines (two CNS cancer cell lines (SF-268, SF-295), a lung cancer cell line (A-549), a breast cancer cell line (MDA-MB-231), and a colon cancer cell line (HCT-116)).[11]

This cyclodepsipeptide can be considered a good model to explore the potential of the triazene linkage strategy as it contains two Phe residues that can be used as anchoring points. The presence of an ester bond on its structure allows also testing the compatibility of this sensitive function with the reaction conditions used for peptide derivatization on the cleavage step.

Another advantage of this strategy is the possibility to perform the cyclization on the solid support if the adequate protecting group scheme is used. In our particular case, the Fmoc group for the amino function and the allyl group for the carboxylic acid function[12] were chosen due to the stability of triazene to the basic (piperidine)[13] and neutral (Pd-0)[14] conditions needed respectively for the removal of these groups.

The protected amino acid Fmoc-Phe(pNH$_2$)-OAllyl (**2b**) was selected to be introduced onto the solid support via triazene linkage. This building block was prepared as described previously.[9] A 4-methyl-benzhydrylamine (MBHA)-polystyrene resin (**2c**) (0.63 mmol·g^{-1}) was functionalized with a glycine residue (internal reference amino acid) in order to control the degree of peptide loading. Then, 4-piperidinecarboxylic acid (isonipecotic acid) residue was added, thereby presenting a secondary amine (Scheme 1).

Compound **2b** was coupled to the modified resin according to the conditions described by Bräse et al.[6b,9] to afford resin **3** (Scheme 2). Spectrophotometric quantification of the Fmoc group[15] indicated resin loading in the 0.34−0.42 mmol·g^{-1} range, reflecting a 75−91% yield for the derivatization process.

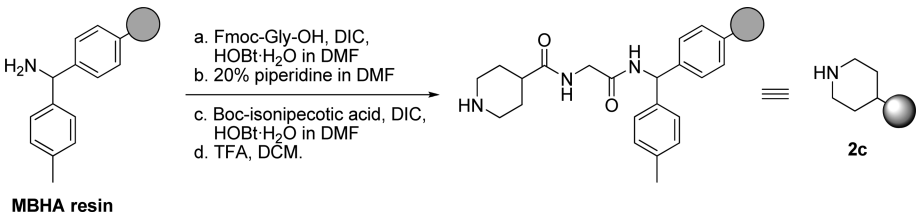
Resin **3** was used to seek appropriate triazene cleavage conditions and concomitant transformation of the resulting diazonium salt to yield the desired derivative. The substitution groups considered in this study were OH, OMe and F, generating Phe derivatives which have been commonly used in SAR studies.[16,17] I and N_3 were also explored because they can be precursors for further modifications under mild conditions using cross coupling processes[18] or click chemistry strategies.[19] As a control, the unmodified protected phenylalanine derivative was obtained under the conditions already established in our laboratory for this substrate (**4a**, Table 1).

For the introduction of hydroxyl, iodo and azido groups, the amino acid was cleaved from the resin as a diazonium salt with diluted TFA in DCM (5:95) and subsequently treated with the corresponding suitable reagent (Table 1). Thus, transformation to Tyr was achieved by treatment with H_2O/CH_3CN (8/2, v/v) at 60 °C. Iodination was performed using KI in H_2O/CH_3CN (9:1)[20] yielding the 4-iodophenylalanine derivative together with the reduced derivative (27%) and derivatization to azide was accomplished with Me_3SiN_3.[21] Methoxy group introduction at the aromatic ring proceeded by resin treatment with TFA at 60 °C using MeOH as solvent instead of DCM.[7b] Finally, fluorine derivative was achieved with yields over 80% treating the resin with $BF_3{\cdot}Et_2O$ in CCl_4 at 80 °C.[22] Other reagents to introduce fluorine were explored such as HF/pyridine after TFA treatment,[23] CsF/C_6F_{14} in TFA[8a,24] or CF_3SO_3H,[24] CsF/(CF_3CH_2OH or $(CF_3)_2CHOH$) in CF_3SO_3H,[24] or AgF/C_6F_{14} in TFA[25] affording low cleavage yields, complex crude product mixtures, or variable quantities of the hydroxyl derivative **4b** (7−83%) as a side product. Phenylalanine derivatives **4a**−**4f** were obtained in 44−78% yields after chromatographic purification. These methodologies proved to be reproducible but anhydrous reagents should be used in order to avoid hydroxylation when other substituent is required.

The solid-phase synthesis of zygosporamide derivatives was planned with final on-resin macrolactamization between Phe^5 and Leu,^{4} thus being Phe^5 the amino acid anchored to the resin through triazene linkage (Scheme 3).

After removal of the Fmoc protecting group of resin **3** with 3% of DBU in DMF, stepwise elongation with standard building blocks (α-hydroxyisocaproic acid, Fmoc-L-Phe-OH, Fmoc-L-Leu-OH and Fmoc-D-Leu-OH) was conducted with diisopropylcarbodiimide. Hydroxybenzotriazole (HOBt) was used as an additive for all amide bond formations. In the case of the ester linkage to the α-hydroxyisocaproic acid residue, 1.5% mol of DMAP was used as an additive instead of HOBt. This procedure rendered resin **5**. After removal of *C*-terminal allyl ester with Pd(PPh$_3$)$_4$/PhSiH$_3$ in DCM and *N*-terminal Fmoc group under the above-mentioned conditions, the peptide backbone underwent quantitative N to C cyclization reaction with PyBOP/HOAt/DIEA in DMF to afford **6**. Acidolytic

Scheme 1. Functionalization of the MBHA Resin



dx.doi.org/10.1021/jo501830w | J. Org. Chem. 2014, 79, 11409−11415

Scheme 2. Coupling of Protected Phenylalanine to the Piperidine Resin via Triazene Linkage

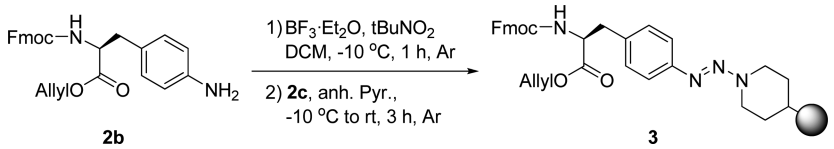
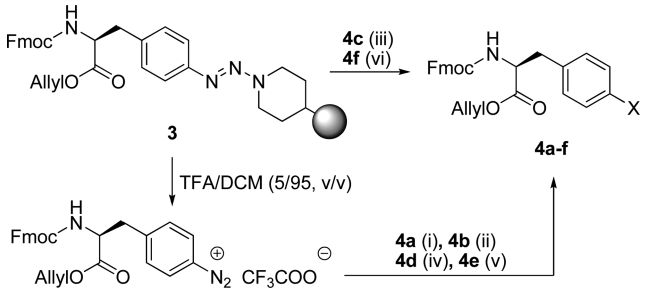


Table 1. Cleavage and Derivatization of Phenylalanine Derivatives



entry	X[a]	% yield[b]
4a	H	66
4b	OH	61
4c	OMe	55
4d	I	44
4e	N_3	78
4f	F	63

[a](i) $FeSO_4·7H_2O$/DMF; (ii) H_2O/CH_3CN (8/2, v/v), 60 °C; (iii) TFA/MeOH (5/95, v/v), 60 °C; (iv) KI, H_2O/CH_3CN (9/1, v/v); (v) Me_3SiN_3; (vi) CCl_4/ $BF_3·Et_2O$, 80 °C. [b]Overall yield after purification.

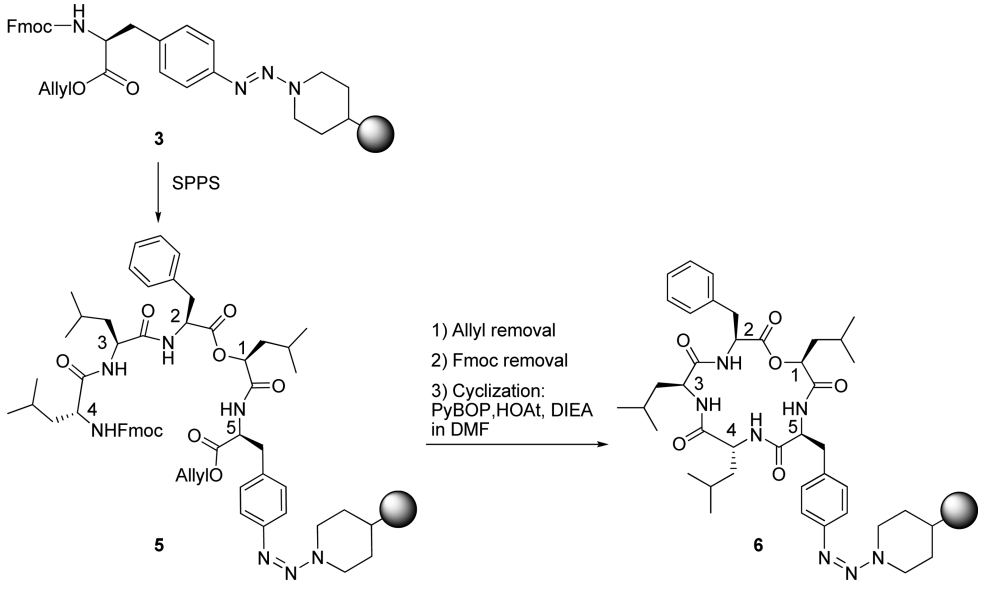
cleavage under reducing conditions[9] led to a 44% overall yield of zygosporamide (**1a**) with a ^{1}H NMR spectrum in good agreement with that reported in the literature.[10] An alternative synthesis of zygosporamide in which Phe[2] was the amino acid anchored to the resin through triazene linkage was attempted unsuccessfully. In this case, stepwise elongation afforded the desired linear peptide; however, the final macrolactonization could not be carried out in any of the conditions tested (data not shown).

Once the synthetic approach for the assembly of the zygosporamide skeleton on the solid support was established, we explored the feasibility of the triazene linkage for the preparation of 4-substituted Phe[5] analogues of this cyclodepsipeptide using the experimental conditions optimized for the resin model **3**. Thus, cleavage of the peptide from the resin with TFA and further treatment with H_2O/CH_3CN at 60 °C afforded **1b** in a 34% overall yield, while similar conditions in the presence of KI at room temperature gave **1d** in a 35% overall yield. Derivative **1c** was obtained when the peptide-resin was submitted to TFA treatment in MeOH (25% overall yield) and azide substitution (**1e**) resulted from acidolytic cleavage of the peptide from the resin followed by reaction of the resulting diazonium salt with Me_3SiN_3 to yield the desired derivative in a 69% global yield. Finally, the fluoride **1f** was obtained when the peptide-resin was treated with a mixture of CCl_4 and $BF_3·Et_2O$ at 80 °C under argon atmosphere (50% overall yield). All yields correspond to the final product after chromatographic purification (Table 2). The identity of the peptide derivatives was confirmed by ^{1}H NMR and/or HRMS.

CONCLUSIONS

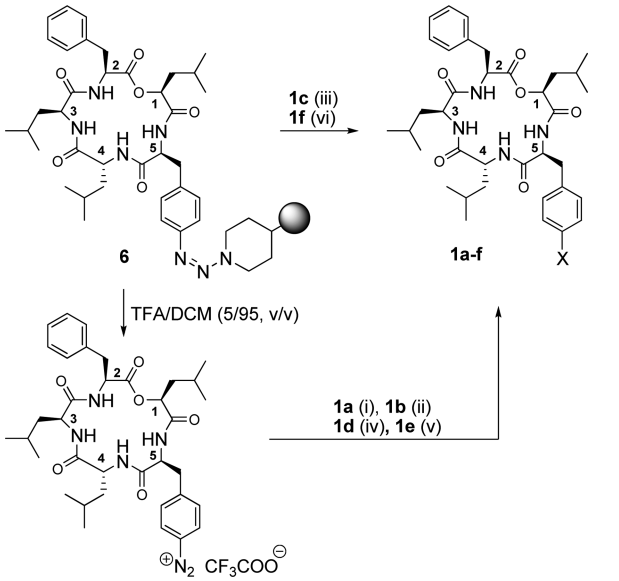
In summary, we have developed a new versatile and efficient method for the solid-phase synthesis of phenylalanine containing peptides, based on the anchorage of the aromatic ring of Phe side chain to the solid support through a triazene linkage. The use of low percentatge of TFA for peptide cleavage from the resin and further chemical transformation of the resulting diazonium salt under suitable experimental conditions allows the introduction of diversity at the *para* position of the

Scheme 3. Solid Phase Synthesis of Zygosporamide Derivatives



dx.doi.org/10.1021/jo501830w | J. Org. Chem. 2014, 79, 11409–11415

Table 2. Cleavage and Derivatization of Zygosporamide Derivatives



entry	X[a]	% yield[b]
1a	H	44
1b	OH	34
1c	OMe	25
1d	I	35
1e	N_3	69
1f	F	50

[a](i) $FeSO_4·7H_2O$/DMF; (ii) H_2O/CH_3CN (8/2, v/v), 60 °C; (iii) TFA/MeOH (5/95, v/v), 60 °C; (iv) KI, H_2O/CH_3CN (9/1, v/v); (v) Me_3SiN_3; (vi) CCl_4/ $BF_3·Et_2O$, 80 °C. [b]Overall yield after purification.

aromatic ring. It is remarkable that this strategy can be also used for the generation of cyclodepsipeptide derivatives due to the mild conditions used, which preserve the integrity of the ester bond. This strategy could be extended to the synthesis of other Phe containing peptide analogues. Furthermore, the high versatility in terms of commercial availability of building blocks for peptide synthesis (orthogonal protecting groups) makes this strategy compatible with the presence of trifunctional amino acids in the peptidic sequence. In this case, the side chain protecting groups should be removed after Phe derivatization.

EXPERIMENTAL SECTION

Reagents and solvents were obtained commercially. Solid-phase peptide synthesis was carried out in polypropylene syringes fitted with porous polystyrene frits. Solvents and excess of reagents were removed by filtration under reduced pressure.

Flash chromatography was performed using an automated flash system. Semipreparative HPLC was carried out with a XBridge BEH 130 C18, 19 × 100 mm column. Elution system used in semipreparative purification HPLC was A: H_2O:CF_3COOH (99.9:0.1, v/v) and B: CH_3CN: CF_3COOH (99.9:0.1, v/v). XSelect-CSH C18 analytical column, 3.5 μm, 4.6 × 50 mm and XBridge BEH 130 C18 analytical column, 3.5 μm, 4.6 × 100 mm columns were used for analytical HPLC-MS. Elution system used in analytical HPLC-MS was A: H_2O:HCOOH (99.9:0.1, v/v) and B: CH_3CN:HCOOH (99.93:0.07, v/v).

NMR spectra were recorded at 400 and 500 MHz spectrometers (^1H and ^{13}C). Mass spectra were acquired with quadrupole detection and an electrospray ion source in positive-ion mode.

(*S*)-Fmoc-Phe(4-NO_2)-OAllyl (2a). (*S*)-Fmoc-Phe(4-NO_2)-OH (3.54 g, 8.19 mmol) was dissolved in DMF (70 mL) and $NaHCO_3$ (3.09 g, 36.79 mmol) and allyl bromide (3.0 mL, 33.63 mmol) were added. The mixture was stirred at rt for 16 h and then the solvent was removed. The pale yellow solid obtained was dissolved in EtOAc (60 mL) and washed with H_2O (3 × 60 mL). The organic phase was dried and solvent removed under a vacuum, furnishing the product as a white solid that was used in the next step without further purification (3.40 g, 88%): mp = 143−146 °C; R_f 0.47 [*t*BuOMe/hexanes (1:1)]; $[\alpha]^D_{20}$ = +15.6 (*c* 1, $CHCl_3$,); IR (ATR) 3329, 1749, 1687, 1516, 1338, 1263, 1213 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.56 (m, 2H), 7.41 (m, 2H), 7.30 (m, 2H), 7.22 (d, *J* = 8.3 Hz, 2H), 5.86 (m, 1H), 5.31 (m, 3H), 4.70 (m, 1H), 4.62 (d, *J* = 5.7 Hz, 2H), 4.51 (dd, J_1 = 10.9 Hz, J_2 = 6.9 Hz, 1H), 4.40 (dd, J_1 = 10.7 Hz, J_2 = 6.4 Hz, 1H), 4.19 (t, *J* = 6.4 Hz, 1H), 3.27 (dd, J_1 = 13.9 Hz, J_2 = 5.8 Hz, 1H), 3.16 (dd, J_1 = 13.8 Hz, J_2 = 5.9 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 155.6, 147.3, 143.8, 143.7, 143.6, 141.5 (× 2), 131.1, 130.4 (× 2), 127.9 (× 2), 127.2 (× 2), 125.0 (× 2), 123.8 (× 2), 120.2 (× 2), 119.8, 66.9, 66.6, 54.6, 47.3, 38.2 ppm; ESI-HRMS calcd for $C_{27}H_{24}N_2O_6Na$ $[M + Na]^+$ 495.1526, found 495.1527.

(*S*)-Fmoc-Phe(4-NH_2)-OAllyl (2b). (*S*)-Fmoc-Phe(4-NO_2)-OAllyl (3.40 g, 7.20 mmol) and dust Zn (2.24 g, 34.26 mmol) were suspended in absolute EtOH (70 mL). AcOH (70 mL) was added to the mixture and the resulting suspension was stirred at 60 °C for 1 h. Solvent was evaporated and product was purified by silica chomatography using DCM/EtOAc (9:1) as eluents, affording the product **2b** as a white solid (2.58 g, 81%): mp = 124−127 °C; R_f 0.41 [DCM/EtOAc (9:1)]; $[\alpha]^D_{20}$ = +15.2 (*c* 1, $CHCl_3$,); IR (ATR) 3389, 2931, 1740, 1692, 1260 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.56 (t, *J* = 6.5 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.30 (tt, J_1 = 7.4 Hz, J_2 = 1.4 Hz, 2H), 6.88 (d, *J* = 8.2 Hz, 2H), 6.58 (d, *J* = 8.2 Hz, 2H), 5.88 (m, 1H), 5.33 (s, 1H), 5.26 (m, 2H), 4.62 (t, J = 6 Hz, 3H), 4.41 (dd, J_1 = 10.6 Hz, J_2 = 7.2 Hz, 1H), 4.33 (dd, J_1 = 10.5 Hz, J_2 = 7.0 Hz, 1H), 4.20 (t, *J* = 7.1 Hz, 1H), 3.56 (bs, 2H), 3.01 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 155.7, 145.6, 144.0, 143.9 (× 2), 141.4 (× 2), 131.6, 130.3 (× 2), 127.8 (× 2), 127.2 (× 2), 125.3, 125.2, 120.1 (× 2), 119.1, 115.4 (× 2), 67.1, 66.1, 55.1, 47.3, 37.5 ppm; ESI-HRMS calcd for $C_{27}H_{27}N_2O_4$ $[M + H]^+$443.1965, found 443.1962.

Isonipecotic-MBHA Resin (2c). MBHA resin (2.60 g, 0.63 mmol/g, 1.64 mmol) was introduced into a polypropylene syringe fitted with a porous polystyrene frit and was washed successively with DCM (10 × 30 s), TFA (40% v/v) in DCM (1 × 1 min and 2 × 10 min), DCM (5 × 30 s), DIEA (5% v/v) in DCM (5 × 2 min), DCM (5 × 30 s) and DMF (5 × 30 s). Then, Fmoc-Gly-OH (internal reference) (1.46 g, 4.91 mmol), HOBt·H_2O (0.75 g, 4.92 mmol) and DIC (0.8 mL, 5.17 mmol) in DMF (8 mL) were added. After 1 h of reaction at rt, the suspension was filtered and the resin was washed with DMF (5 × 30 s), DCM (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s). Then, Fmoc group was removed with 20% piperidine in DMF (1 × 1 min, 2 × 10 min) and Boc-isonipecotic acid (1.13 g, 4.92 mmol), HOBt·H_2O (0.75 g, 4.92 mmol) and DIC (0.8 mL, 5.17 mmol) in DMF (8 mL) were added. After 1 h at rt, the mixture was washed with DMF (5 × 30 s), DCM (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s). Finally, the resin was treated with 40% of TFA in DCM to remove Boc group (2 × 10 min), and was washed with DCM (5 × 30 s), MeOH (5 × 30 s), DCM (5 × 30 s) and DMF (5 × 30 s).

Phenylalanine Incorporation to Resin 2c via Triazene Linkage (3). Compound **2b** (2.56 g, 5.10 mmol) was dissolved under Ar in anhydrous DCM (40 mL) and the resulting solution was cooled down to −10 °C, then $BF_3·Et_2O$ (1.30 mL, 10.3 mmol) and *t*$BuNO_2$ (1.40 mL, 10.6 mmol) were added. The mixture was stirred at this temperature under Ar atmosphere for 1 h and it was added via cannula to a mixture of the isonipecotic-MBHA resin (3.15 g, 1.70 mmol) and anhydrous pyridine (12 mL, 0.15 mol) at −10 °C. The resulting suspension was shaken at rt for 3 h under Ar atmosphere and filtered. Then, the resin was washed with DCM (5 × 30 s), MeOH (5 × 30 s), DCM (5 × 30 s) and DMF (5 × 30 s). Spectrophotometric quantification of Fmoc groups afforded an 80% yield of phenylalanine

dx.doi.org/10.1021/jo501830w | J. Org. Chem. 2014, 79, 11409−11415

coupling through triazene linker. Finally, the unreacted secondary amide groups were capped by treatment with Ac_2O (3.22 mL, 34.1 mmol) and DIEA (5.90 mL, 33.9 mmol) in DMF (6 mL) for 30 min. Then, the mixture was filtered and resin washed with DMF (5 × 30 s), DCM (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s) affording the resin **3**.

Fmoc-Phe-OAllyl (4a). Resin **3** (161 mg, 0.10 mmol) was cleaved by treatment with TFA in DCM (5/95 v/v; 3 × 2 min), and the collected washings were evaporated under a vacuum to dryness (the diazonium salt derivative was protected from light). Then, the diazonium salt derivative was dissolved in DMF (5 mL) and $FeSO_4 \cdot 7H_2O$ (0.04 g, 0.14 mmol) was added. The mixture was stirred at rt for 5 min and the solvent was removed under a vacuum, affording the amino acid crude (84% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 83% in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 6.0 min), yielding 15 mg of **4a** (global yield of 66%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.59−7.52 (m, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.34−7.24 (m, 5H), 7.11 (d, J = 6.5 Hz, 2H), 5.93−5.81 (m, 1H), 5.33 (bs, 1H), 5.30−5.21 (m, 2H), 4.74−4.66 (m, 1H), 4.62 (d, J = 5.8 Hz, 2H), 4.44 (dd, J = 10.6, 7.1 Hz, 1H), 4.34 (dd, J = 10.6, 7.0 Hz, 1H), 4.21 (t, J = 7.1 Hz, 1H), 3.20−3.07 (m, 2H);^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 155.7, 144.0 (× 2), 141.5 (× 2), 135.8, 131.5, 129.5 (× 2), 128.8 (× 2), 127.9 (× 2), 127.3, 127.2 (× 2), 125.3 (× 2), 120.1 (× 2), 119.3, 67.1, 66.3, 55.0, 47.3, 38.4 ppm; ESI-HRMS calcd for $C_{27}H_{26}NO_4$ $[M + H]^+$ 428.1856, found 428.1862.

Fmoc-Phe(4-OH)-OAllyl (4b). Resin **3** (213 mg, 0.13 mmol) was cleaved by treatment with TFA in DCM (5/95 v/v; 3 × 2 min), and the collected washings were evaporated under a vacuum to dryness (the diazonium salt derivative was protected from light). Then, the diazonium salt was dissolved in H_2O/CH_3CN (8/2, v/v). The mixture was stirred at 60 °C for 2.5 h and the solvent was removed under a vacuum, affording the amino acid crude (71% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 78% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 4.27 min), yielding 19 mg of **4b** (global yield of 61%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.56 (dd, J_1 = 7.1 Hz, J_2 = 3.9 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 6.95 (d, J = 8.2 Hz, 2H), 6.72 (d, J = 8.3 Hz, 2H), 5.93−5.80 (m, 1H), 5.34 (s, 1H), 5.31−5.21 (m, 2H), 4.69−4.63 (m, 1H), 4.62 (d, J = 5.8, 2H), 4.47−4.31 (m, 2H), 4.20 (t, J = 7.0 Hz, 1H), 3.12−2.98 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 155.8, 155.1, 143.8 (× 2), 141.5 (× 2), 131.5, 130.7 (× 2), 127.9 (× 2), 127.6, 127.2 (× 2), 125.2 (× 2), 120.1 (× 2), 119.4, 115.6 (× 2), 67.2, 66.3, 55.1, 47.3, 37.6 ppm; ESI-HRMS calcd for $C_{27}H_{26}NO_5$ $[M + H]^+$ 444.1805, found 444.1806.

Fmoc-Phe(4-OMe)-OAllyl (4c). Resin **3** (250 mg, 0.17 mmol) was treated with TFA in MeOH (5/95 v/v) at 60 °C for 1 h. The resin was filtered and the corresponding solution was evaporated under a vacuum to dryness, affording the amino acid crude (77% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 87% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 5.65 min), yielding 21 mg of **4c** (global yield of 55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.56 (t, J = 6.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 5.94−5.82 (m, 1H), 5.34 (s, 1H), 5.31−5.20 (m, 2H), 4.69−4.64 (m, 1H), 4.62 (d, J = 5.7, 2H), 4.44 (dd, J_1 = 10.6 Hz, J_2 = 7.1 Hz, 1H), 4.34 (dd, J_1 = 10.5 Hz, J_2 = 7.0 Hz, 1H), 4.21 (t, J = 7.0 Hz, 1H), 3.77 (s, 3H), 3.14−3.01 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 158.9, 155.7, 144.0 (× 2), 141.5 (× 2), 131.6, 130.5 (× 2), 127.9 (× 2), 127.7, 127.2 (× 2), 125.2 (× 2), 120.1 (× 2), 119.3, 114.2 (× 2), 67.1, 66.2, 55.4, 55.06, 47.3, 37.5 ppm; ESI-HRMS calcd for $C_{28}H_{28}NO_5$ $[M + H]^+$ 458.1962, found 458.1967.

Fmoc-Phe(4-I)-OAllyl (4d). Resin **3** (130 mg, 0.08 mmol) was treated with TFA in DCM (5/95 v/v; 3 × 2 min), and the collected washings were evaporated under a vacuum to dryness (the diazonium salt derivative was protected from light). Then, the diazonium salt was dissolved in H_2O/CH_3CN (9/1, v/v) and KI (36 mg, 0.22 mmol) was added. The mixture was stirred at rt for 1 h and the solvent was removed under a vacuum, affording the amino acid crude (53% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 93% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 6.92 min), yielding 10 mg of **4d** (global yield of 44%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.63−7.53 (m, 4H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 6.83 (d, J = 8.0 Hz, 2H), 5.93−5.79 (m, 1H), 5.34 (s, 1H), 5.30−5.20 (m, 2H), 4.70−4.64 (m, 1H), 4.61 (d, J = 5.6, 2H), 4.47 (dd, J_1 =10.7 Hz, J_2 = 7.3 Hz, 1H), 4.37 (dd, J_1 = 10.6 Hz, J_2 = 6.5 Hz, 1H), 4.21 (t, J = 6.7 Hz, 1H), 3.14−3.00 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 155.7, 143.8 (× 2), 141.5 (× 2), 137.8 (× 2), 135.4, 131.6 (× 2), 131.4, 127.9 (× 2), 127.3 (× 2), 125.2 (× 2), 120.2 (× 2), 119.6, 92.7, 67.2, 66.4, 54.8, 47.4, 37.9 ppm; ESI-HRMS calcd for $C_{27}H_{25}INO_4$ $[M + H]^+$ 554.0823, found 554.0817.

Fmoc-Phe(4-N_3)-OAllyl (4e). Resin **3** (110 mg, 0.07 mmol) was treated with TFA in DCM (5/95 v/v) during 10 min and then, Me_3SiN_3 (40 μL, 0.29 mmol) was added. The mixture was stirred at rt for 1 h and the solvent was removed under a vacuum, affording the amino acid crude (94% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 70% B in 1 min and 70% → 84% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 6.0 min), yielding 13 mg of **4e** (global yield of 78%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.60−7.52 (m, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 7.06 (d, J = 8.2 Hz, 2H), 6.93 (d, J = 8.3 Hz, 2H), 5.94−5.82 (m, 1H), 5.34 (s, 1H), 5.32−5.20 (m, 2H), 4.71−4.64 (m, 1H), 4.62 (d, J = 5.8, 2H), 4.47 (dd, J_1 = 10.6 Hz, J_2 = 7.1 Hz, 1H), 4.37 (dd, J_1 = 10.5 Hz, J_2 = 6.8 Hz, 1H), 4.20 (t, J = 6.8 Hz, 1H), 3.18−3.02 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 155.6, 144.0, 143.8, 141.5 (× 2), 139.1,132.5, 131.4, 130.9 (× 2), 127.9 (× 2), 127.2 (× 2), 125.2, 125.1, 120.2 (× 2), 119.5, 119.3 (× 2), 67.0, 66.4, 54.9, 47.3, 37.8 ppm; ESI-HRMS calcd for $C_{27}H_{25}N_4O_4$ $[M + H]^+$ 469.1870, found 469.1877.

Fmoc-Phe(4-F)-OAllyl (4f). Resin **3** (185 mg, 0.12 mmol), CCl_4 (3 mL) and $BF_3 \cdot Et_2O$ (0.1 mL, 0.75 mmol) were introduced in a sealed tube under argon atmosphere. After 5 min at rt, the mixture was heated at 80 °C for 1.5 h. Finally, the resin was filtered and washed with CH_3CN. Then, the collected washings were evaporated under a vacuum to dryness, affording the amino acid crude (90% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 90% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 5.73 min), yielding 18 mg of **4f** (global yield of 63%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.59−7.52 (m, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 7.09−7.01 (m, 2H), 7.00−6.91 (m, 2H), 5.92−5.81 (m, 1H), 5.33 (s, 1H), 5.26 (dd, J_1 = 15.8 Hz, J_2 = 5.7 Hz, 2H), 4.66 (dd, J_1 = 14.1 Hz, J_2 = 6.3 Hz, 1H), 4.61 (d, J = 5.8, 2H), 4.47 (dd, J_1 = 10.6 Hz, J_2 = 7.2 Hz, 1H), 4.37 (dd, J_1 = 10.5 Hz, J_2 = 6.8 Hz, 1H), 4.20 (t, J = 6.8 Hz, 1H), 3.17−3.01 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 162.5 (J_{C-F} = 245.5 Hz), 155.9, 144.3 (× 2), 141.8 (× 2), 131.8, 131.7, 131.4, 131.3, 128.2 (× 2), 127.5 (× 2), 125.5, 125.4, 120.5 (× 2), 119.8, 116.0, 115.8, 67.3, 66.7, 55.3, 47.6, 37.9 ppm; ^{19}F (376 MHz, $CDCl_3$) δ −115.6 ppm; ESI-HRMS calcd for $C_{27}H_{25}FNO_4$ $[M + H]^+$ 446.1762, found 446.1769.

General Procedures for Solid-Phase Peptide Synthesis. *Amino Acid Coupling.* To the resin were added the amino acid (3 equiv), $HOBt \cdot H_2O$ (3 equiv) and DIC (3 equiv) in DMF (5−7 mL) and the mixture reacted for 1 h at rt with occasional manual stirring. Then, the resin was filtered and washed with DMF (5 × 30 s), DCM (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s). Couplings were monitored until completeness using the Kaiser test.

Removal of the Fmoc Group. The resin was treated with 20% piperidine in DMF (v/v, 1 × 1 min and 2 × 10 min) and then it was washed with DMF (5 × 30 s), DCM (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s). In the synthesis of zygosporamide, after the step of ester formation, the Fmoc groups were removed by treatment of the resin with 3% DBU in DMF (v/v, 1 × 1 min and 2 × 10 min) and then it was washed with DMF (5 × 30 s), DCM (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s).

dx.doi.org/10.1021/jo501830w | J. Org. Chem. 2014, 79, 11409−11415

Removal of Allyl Group. The resin was washed with DMF (5 × 30 s) and DCM (5 × 30 s). Then, it was suspended in DCM and degassed by bubbling Ar for 5 min, when $Pd(PPh_3)_4$ (0.4 equiv) and $PhSiH_3$ (48 equiv) in DCM (8 mL) were added. The mixture was shaken for 30 min at rt, filtered and washed with DCM (8 × 30 s). This treatment was carried out twice under the same conditions. After filtration, the resin was washed with DCM (8 × 30 s), a solution of sodium diethyl dithiocarbamate (5% v/v) in DMF (2 × 5 min), DMF (5 × 1 min) and DCM (5 × 30 s).

Peptidyl Resin ***6.*** After removal of the Fmoc group of resin **3** (1.39 g, 0.91 mmol), α-hydroxyisocaproic acid was incorporated using conditions from general procedure A. The best conditions for ester bond with Phe^2 were as follows: Fmoc-Phe-OH (7 equiv), DIC (7 equiv) and DMAP (0.1 equiv) in DCM (15 mL) were added to the resin and the mixture reacted for 1 h at rt with occasional manual stirring. Then, the resin was filtered and washed with DCM (5 × 30 s), DMF (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s). According to HPLC-MS analysis, the desired tripeptide was obtained in 95% yield together with 5% of epimerized product. The subsequent amino acids Leu^3 and Leu^4 were assembled following the general procedures for solid-phase peptide synthesis, affording resin **5**. Then, the *C*-terminal allyl group and *N*-terminal Fmoc group were removed following the general procedures previously described (using 3% DBU in DMF) respectively, affording the unprotected linear peptide on the solid support. Cyclization was carried out with PyBOP (1.90 g, 3.65 mmol), HOAt (0.5 g, 3.67 mmol) and DIEA (1.3 mL, 7.46 mmol) in DMF (15 mL) for 2 h at rt. Then, the resin was washed with DCM (5 × 30 s), DMF (5 × 30 s), MeOH (5 × 30 s) and DCM (5 × 30 s). The resulting peptidyl resin **6** was used to obtain different derivatives of zygosporamide.

*Zygosporamide X = H (**1a**).* Peptidyl resin (270 mg, 0.09 mmol) **6** was cleaved with TFA in DCM (5/95 v/v; 3 × 2 min), and the collected washings were evaporated under a vacuum to dryness (the diazonium salt peptide derivative was protected from light). Then, the diazonium salt peptide derivative was dissolved in DMF (5 mL) and $FeSO_4{\cdot}7H_2O$ (0.04 g, 0.14 mmol) was added. The mixture was stirred at rt for 5 min and the solvent was removed under a vacuum, affording peptide crude (55% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 76% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 5.8 min), yielding 25 mg of **1a** (global yield of 44%): ESI-HRMS calcd for $C_{36}H_{51}N_4O_6$ $[M + H]^+$, 635.3803, found 635.3798; ^{1}H NMR (500 MHz, CD_3CN) δ 7.61 (d, J = 9.6 Hz, 1H), 7.35−7.18 (m, 11H), 7.12 (d, J = 5.9 Hz, 1H), 6.96 (d, J = 9.3 Hz, 1H), 4.84 (ddd, J_1 = 11.0 Hz, J_2 = 10.0 Hz, J_3 = 5.0, 1H), 4.74 (dd, J_1 = 9.3 Hz, J_2 = 5.1 Hz, 1H), 4.63−4.57 (m, 1H), 4.16−4.05 (m, 2H), 3.33 (dd, J_1 = 14.0 Hz, J_2 = 4.9 Hz, 1H), 3.11 (dd, J_1 = 14.0 Hz, J_2 = 11.1 Hz, 1H), 3.05 (dd, J_1 = 13.6 Hz, J_2 = 5.8 Hz, 1H), 2.85 (dd, J_1 = 13.6 Hz, J_2 = 9.2 Hz, 1H), 1.76−1.68 (m, 1H), 1.59−1.35 (m, 6H), 1.27−1.21 (m, 2H), 0.91 (dd, J_1 = 7.9 Hz, J_2 = 6.5 Hz, 6H), 0.88−0.83 (m, 9H), 0.77 (d, J = 6.6 Hz, 3H) ppm.

*Zygosporamide X = OH (**1b**).* Peptidyl resin **6** (280 mg, 0.10 mmol) was cleaved with TFA in DCM (5/95 v/v; 3 × 2 min), and the collected washings were evaporated under a vacuum to dryness (the diazonium salt peptide derivative was protected from light). Then, the diazonium salt peptide derivative was dissolved in H_2O/CH_3CN (8/2, v/v). The mixture was stirred at 60 °C for 2 h and the solvent was removed under a vacuum, affording the peptide crude (64% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 60% B in 1 min and 60% → 62% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 4.65 min), yielding 21 mg of **1b** (global yield of 34%): ESI-HRMS calcd for $C_{36}H_{51}N_4O_7$ $[M + H]^+$, 651.3752, found 651.3743; ^{1}H NMR (500 MHz, CD_3CN) δ 7.57 (d, J = 9.7 Hz, 1H), 7.33−7.25 (m, 4H), 7.25−7.20 (m, 1H), 7.15 (d, J = 8.3 Hz, 1H), 7.09−7.02 (m, 3H), 6.87 (d, J = 9.5 Hz, 1H), 6.74−6.70 (m, 2H), 4.83 (ddd, J_1 = 11.0 Hz, J_2 = 9.8 Hz, J_3 = 5.0, 1H), 4.74 (dd, J_1 = 9.4 Hz, J_2 = 5.1 Hz, 1H), 4.50 (td, J_1 = 9.1 Hz, J_2 = 6.2 Hz, 1H), 4.14−4.02 (m, 2H), 3.33 (dd, J_1 = 14.0 Hz, J_2 = 5.0 Hz, 1H), 3.10 (dd, J_1 = 14.0 Hz, J_2 = 11.1 Hz, 1H), 2.92 (dd, J_1 = 13.7 Hz, J_2 = 6.2 Hz, 1H), 2.75 (dd, J_1 = 13.7 Hz, J_2 = 8.9 Hz, 1H), 1.73 (ddd, J_1 = 13.4 Hz, J_2 = 9.4 Hz, J_3 = 5.5, 1H), 1.59−1.45 (m, 4H), 1.45−1.35 (m, 2H), 1.25−1.20 (m, 2H), 0.92 (dd, J_1 = 6.4 Hz, J_2 = 1.4 Hz, 6H), 0.89−0.82 (m, 9H), 0.76 (d, J = 6.6 Hz, 3H) ppm.

*Zygosporamide X = OMe (**1c**).* Peptidyl resin **6** (290 mg, 0.10 mmol) was treated with TFA in MeOH (5/95 v/v) at 60 °C for 1 h. The peptidyl resin was filtered and the corresponding solution was evaporated under a vacuum to dryness, affording the peptide crude (57% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 73% B in 7 min with a flow rate of 16 mL/min and 214 nm UV detection, t_R = 5.60 min), yielding 23 mg of **1c** (global yield of 35%): ESI-HRMS calcd for $C_{37}H_{52}N_4O_7$ $[M + H]^+$, 665.3909, found 665.3906; ^{1}H NMR (500 MHz, CD_3CN) δ 7.76 (bs, 1H), 7.39 (bs, 1H), 7.34−7.25 (m, 4H), 7.25−7.20 (m, 1H), 7.20−7.11 (m, 4H), 6.87−6.81 (m, 2H), 4.81 (bs, 1H), 4.77 (dd, J_1 = 9.1 Hz, J_2 = 5.1 Hz, 1H), 4.62−4.52 (m, 1H), 4.17 (dd, J_1 = 13.4 Hz, J_2 = 6.6 Hz, 1H), 4.11 (ddd, J_1 = 10.6 Hz, J_2 = 8.4 Hz, J_3 = 4.9, 1H), 3.75 (s, 3H), 3.30 (dd, J_1 = 14.0 Hz, J_2 = 5.0 Hz, 1H), 3.17−3.07 (m, 1H), 3.02 (dd, J_1 = 13.5 Hz, J_2 = 5.5 Hz, 1H), 2.85−2.73 (m, 1H), 1.73−1.62 (m, 1H), 1.58−1.18 (m, 8H), 0.93−0.81 (m, 15H), 0.76 (d, J = 6.6 Hz, 3H) ppm.

*Zygosporamide X = I (**1d**).* Peptidyl resin **6** (125 mg, 0.05 mmol) was cleaved with TFA in DCM (5/95 v/v; 3 × 2 min), and the collected washings were evaporated under a vacuum to dryness (the diazonium salt peptide derivative was protected from light). Then, the diazonium salt peptide derivative was dissolved in H_2O/CH_3CN (9/1, v/v) and KI (49.8 mg, 0.30 mmol) was added. The mixture was stirred at rt for 1 h and the solvent was removed under a vacuum, affording the peptide crude (53% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 70% B in 1 min and 70% → 82% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 6.08 min), yielding 8 mg of **1d** (global yield of 25%): ESI-HRMS calcd for $C_{36}H_{50}IN_4O_6$ $[M + H]^+$, 761.2770, found 761.2771; ^{1}H NMR (500 MHz, CD_3CN) δ 7.66 (d, J = 8.2 Hz, 2H), 7.62 (d, J = 9.6 Hz, 1H), 7.33−7.19 (m, 6H), 7.10−7.04 (m, 3H), 7.02 (d, J = 5.9 Hz, 1H), 4.85−4.73 (m, 2H), 4.61−4.53 (m, 1H), 4.18−4.06 (m, 2H), 3.30 (dd, J_1 = 14.0 Hz, J_2 = 5.0 Hz, 1H), 3.10 (dd, J_1 = 14.0 Hz, J_2 = 11.1 Hz, 1H), 3.02 (dd, J_1 = 13.5 Hz, J_2 = 6.0 Hz, 1H), 2.80 (dd, J_1 = 13.6 Hz, J_2 = 9.4 Hz, 1H), 1.72−1.62 (m, 1H), 1.58−1.32 (m, 6H), 1.32−1.17 (m, 2H), 0.91 (dd, J_1 = 11.5 Hz, J_2 = 6.3 Hz, 6H), 0.88−0.82 (m, 9H), 0.76 (d, J = 6.6 Hz, 3H) ppm.

*Zygosporamide X = N_3 (**1e**).* Peptidyl resin **6** (175 mg, 0.06 mmol) was treated with TFA in DCM (5/95 v/v; 10 min) and then Me_3SiN_3 (68 μL, 0.55 mmol) was added. The mixture was stirred at rt for 1 h and the solvent was removed under a vacuum, affording the peptide crude (71% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (30% → 65% B in 1 min and 65% → 77% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R = 6.43 min), yielding 27 mg of **1e** (global yield of 69%): ESI-HRMS calcd for $C_{36}H_{50}N_7O_6$ $[M + H]^+$, 676.3817, found 676.3815; ^{1}H NMR (500 MHz, CD_3CN) δ 7.59 (d, J = 9.7 Hz, 1H), 7.34−7.19 (m, 8H), 7.15 (d, J = 6.1 Hz, 1H), 7.03−6.99 (m, 2H), 6.98 (d, J = 10.7 Hz, 1H), 4.83 (ddd, J_1 = 10.9 Hz, J_2 = 9.9 Hz, J_3 = 5.0, 1H), 4.74 (dd, J_1 = 9.3 Hz, J_2 = 5.1 Hz, 1H), 4.59 (td, J_1 = 9.4 Hz, J_2 = 5.9 Hz, 1H), 4.17−4.02 (m, 2H), 3.33 (dd, J_1 = 14.0 Hz, J_2 = 5.0 Hz, 1H), 3.11 (dd, J_1 = 14.0 Hz, J_2 = 11.1 Hz, 1H), 3.03 (dd, J_1 = 13.6 Hz, J_2 = 5.9 Hz, 1H), 2.83 (dd, J_1 = 13.6 Hz, J_2 = 9.4 Hz, 1H), 1.76−1.68 (m, 1H), 1.57−1.35 (m, 6H), 1.28 (d, J = 5.3 Hz, 1H), 1.26−1.21 (m, 2H), 0.95−0.89 (m, 6H), 0.89−0.82 (m, 9H), 0.76 (d, J = 6.6 Hz, 3H) ppm.

*Zygosporamide X = F (**1f**).* Peptidyl resin **6** (230 mg, 0.08 mmol), CCl_4 (4 mL) and $BF_3{\cdot}Et_2O$ (0.4 mL, 1.51 mmol) were introduced in a sealed tube under argon atmosphere. After 5 min at rt, the mixture was heated at 80 °C for 1.5 h. Finally, the peptidyl resin was filtered and washed with CH_3CN. Then, the collected washings were evaporated under a vacuum to dryness, affording the peptide crude (73% of purity by HPLC) that was purified by semipreparative reversed-phase HPLC (5% → 65% B in 1 min and 65% → 73% B in 7 min with a flow rate of 16 mL/min and λ = 214 nm, t_R =5.92 min), yielding 25 mg of **1f** (global yield of 50%): ESI-HRMS calcd for $C_{36}H_{50}FN_4O_6$ $[M + H]^+$, 653.3709, found 653.3705; ^{1}H NMR (500 MHz, CD_3CN) δ 7.76 (d, J = 8.3 Hz, 1H), 7.36 (d, J = 7.1 Hz, 1H), 7.32−7.24 (m, 6H), 7.24−

dx.doi.org/10.1021/jo501830w | J. Org. Chem. 2014, 79, 11409−11415

7.20 (m, 1H), 7.18 (bs, 1H), 7.08 (bs, 1H), 7.06−7.00 (m, 2H), 4.85−4.75 (m, 2H), 4.63−4.55 (m, 1H), 4.23−4.15 (m, 1H), 4.12 (ddd, J_1 = 10.8 Hz, J_2 = 8.5 Hz, J_3 = 4.9, 1H), 3.29 (dd, J_1 = 14.0 Hz, J_2 = 5.1 Hz, 1H), 3.15−3.05 (m, 2H), 2.83 (dd, J_1 = 13.7 Hz, J_2 = 9.8 Hz, 1H), 1.70−1.61 (m, 1H), 1.60−1.18 (m, 8H), 0.92 (d, J = 6.4 Hz, 3H), 0.90−0.86 (m, 6H), 0.86−0.82 (m, 6H), 0.76 (d, J = 6.6 Hz, 3H) ppm.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of phenylalanine derivatives; ^{1}H and TOCSY NMR spectra of peptides; HPLC-MS data of crude phenylalanine derivatives and peptides; HPLC of pure phenylalanine derivatives and peptides; HRMS of pure phenylalanine derivatives and peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: mroyo@pcb.ub.es.

*E-mail: enicolas@ub.edu.

Present Address

#Health Sciences Center, YachayTech, Imbabura, Ecuador.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Peptide Synthesis Service of the Biomedical Networking Center (CIBER-BBN) and the Parc Cientific of Barcelona (PCB) for their assistance analytical data (HPLC and HPLC-MS). This work was funded by the Spanish government (SAF2011-30508-C02-01 (MR), CTQ2006-12460 (EN)), CIBER-BBN (CB06_01_0074 (FA)) and the Generalitat de Catalunya (2009SGR1024).

■ REFERENCES

(1) (a) Joo, S. H. *Biomol. Ther.* **2012**, *20*, 19−26. (b) Katsara, M.; Tselios, T.; Deraos, S.; Deraos, G.; Matsoukas, M. T.; Lazoura, E.; Matsoukas, J.; Apostolopoulos, V. *Curr. Med. Chem.* **2006**, *13*, 2221−2232. (c) Pomilio, A. B.; Battista, M. E.; Vitale, A. A. *Curr. Org. Chem.* **2006**, *10*, 2075−2121. (d) Hamada, Y.; Shioiri, T. *Chem. Rev.* **2005**, *105*, 4441−4482.

(2) (a) Zhou, P.; Huang, J.; Tian, F. *Curr. Med. Chem.* **2012**, *19*, 226−238. (b) Salonen, L. M.; Ellermann, M.; Diederich, F. *Angew. Chem., Int. Ed.* **2011**, *50*, 4808−4842. (c) Suresh, C. H.; Mohan, N.; Vijayalakshmi, K. P.; George, R.; Mathew, J. M. *J. Comput. Chem.* **2009**, *30*, 1392−1404. (d) Waters, M. L. *Biopolymers* **2004**, *76*, 435−445. (e) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210−1250.

(3) (a) Martin-Gago, P.; Gomez-Caminals, M.; Ramon, R.; Verdaguer, X.; Martin-Malpartida, P.; Aragon, E.; Fernandez-Carneado, J.; Ponsati, B.; Lopez-Ruiz, P.; Cortes, M. A.; Colas, B.; Macias, M. J.; Riera, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 1820−1825. (b) Jalali-Heravi, M.; Mani-Varnosfaderani, A. *SAR QSAR Environ. Res.* **2011**, *22*, 293−314. (c) Solanas, C.; García-de la Torre, B.; Fernandez-Reyes, M.; Santiveri, C. M.; Jimenez, M. A.; Rivas, L.; Jimenez, A. I.; Andreu, D.; Cativiela, C. *J. Med. Chem.* **2010**, *53*, 4119−4129. (d) Szymanska, E.; Pickering, D. S.; Nielsen, B.; Johansen, T. N. *Bioorg. Med. Chem.* **2009**, *17*, 6390−6401. (e) Colombo, A.; Fernandez, J. C.; de la Figuera, N.; Fernandez-Forner, D.; Forns, P.; Albericio, F. *QSAR Comb. Sci.* **2005**, *24*, 913−922. (f) Kamm, W.; Hauptmann, J.; Behrens, I.; Sturzebecher, J.; Dullweber, F.; Gohlke, H.; Stubbs, M.; Klebe, G.; Kissel, T. *Pharm. Res.* **2001**, *18*, 1110−1118. (g) Bonner, G. G.; Davis, P.; Stropova, D.; Edsall, S.; Yamamura, H. I.; Porreca, F.; Hruby, V. J. *J. Med. Chem.* **2000**, *43*, 569−580.

(4) Knepper, K.; Ziegert, R. E. T1 and T2, Versatile Triazene Linker Groups. In *Linker Strategies in Solid-Phase Organic Synthesis*; Scott, P. J. H., Ed.; John Wiley & Sons, Ltd.: Chichester, 2009; pp 263−302.

(5) Bräse, S.; Enders, D.; Kobberling, J.; Avemaria, F. *Angew. Chem., Int. Ed.* **1998**, *37*, 3413−3415.

(6) (a) Bräse, S. *Acc. Chem. Res.* **2004**, *37*, 805−816. (b) Bräse, S.; Dahmen, S.; Lormann Matthias, E. *Methods Enzymol.* **2003**, *369*, 127−150. (c) Gil, C.; Bräse, S. *Curr. Opin. Chem. Biol.* **2004**, *8*, 230−237.

(7) (a) Lormann, M.; Dahmen, S.; Bräse, S. *Tetrahedron Lett.* **2000**, *41*, 3813−3816. (b) Schunk, S.; Enders, D. *J. Org. Chem.* **2002**, *67*, 8034−8042.

(8) (a) Doebele, M.; Vanderheiden, S.; Jung, N.; Bräse, S. *Angew. Chem., Int. Ed.* **2010**, *49*, 5986−5988. (b) Gil, C.; Bräse, S. *Chem.—Eur. J.* **2005**, *11*, 2680−2688.

(9) Torres-Garcia, C.; Pulido, D.; Carceller, M.; Ramos, I.; Royo, M.; Nicolas, E. *J. Org. Chem.* **2012**, *77*, 9852−9858.

(10) Oh, D. C.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **2006**, *47*, 8625−8628.

(11) Wang, Y.; Zhang, F.; Zhang, Y.; Liu, J. O.; Ma, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4385−4387.

(12) Kates, S. A.; Sole, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549−1552.

(13) Sheppard, R. *J. Pept. Sci.* **2003**, *9*, 545−552.

(14) Guibe, F. *Tetrahedron* **1998**, *54*, 2967−3042.

(15) Chan, W. C., White, P. D., Eds.; *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press: Oxford, U.K., 2000.

(16) Kawai, M.; Chorev, M.; Marin-Rose, J.; Goodman, M. *J. Med. Chem.* **1980**, *23*, 420−424.

(17) (a) Salwiczek, M.; Nyakatura, E. K.; Gerling, U. I. M.; Ye, S.; Koksch, B. *Chem. Soc. Rev.* **2012**, *41*, 2135−2171. (b) Vulpetti, A.; Dalvit, C. *Drug Discovery Today* **2012**, *17*, 890−897.

(18) (a) Li, J.; Chen, P. R. *ChemBioChem* **2012**, *13*, 1728−1731. (b) Vilaro, M.; Arsequell, G.; Valencia, G.; Ballesteros, A.; Barluenga, J. *Org. Lett.* **2008**, *10*, 3243−3245. (c) Kodama, K.; Fukuzawa, S.; Nakayama, H.; Sakamoto, K.; Kigawa, T.; Yabuki, T.; Matsuda, N.; Shirouzu, M.; Takio, K.; Yokoyama, S.; Tachibana, K. *ChemBioChem* **2007**, *8*, 232−238. (d) Kodama, K.; Fukuzawa, S.; Nakayama, H.; Kigawa, T.; Sakamoto, K.; Yabuki, T.; Matsuda, N.; Shirouzu, M.; Takio, K.; Tachibana, K.; Yokoyama, S. *ChemBioChem* **2006**, *7*, 134−139. (e) Dibowski, H.; Schmidtchen, F. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 476−478.

(19) (a) Sommer, S.; Weikart, N. D.; Brockmeyer, A.; Janning, P.; Mootz, H. D. *Angew. Chem., Int. Ed.* **2011**, *50*, 9888−9892. (b) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2009**, *48*, 6974−6998.

(20) Filimov, V. D.; Trusova, M.; Postnikov, P.; Krasnokutskaya, E. A.; Lee, Y. M.; Hwang, H. Y.; Kim, H.; Chi, K. W. *Org. Lett.* **2008**, *10*, 3961−3964.

(21) Avemaria, F.; Zimmermann, V.; Bräse, S. *Synlett.* **2004**, *7*, 1163−1166.

(22) Kovac, M.; Anderluh, M.; Vercouillie, J.; Guilloteau, D.; Emond, P.; Mavel, S. *J. Fluorine Chem.* **2013**, *147*, 5−9.

(23) Fukuhara, T.; Yoneda, N.; Sawada, T.; Suzuki, A. *Synth. Commun.* **1987**, *17*, 685−692.

(24) Pages, T.; Langlois, B. R. *J. Fluorine Chem.* **2001**, *107*, 321−327.

(25) Haroutounian, S. A.; Dizio, J. P.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *56*, 4993−4996.