Organic LETTERS

Letter

pubs.acs.org/OrgLett

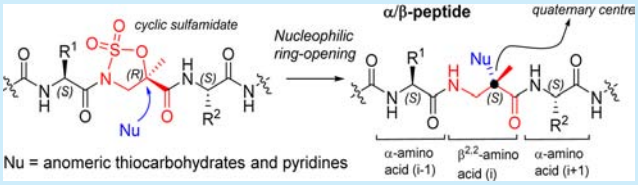
Synthesis of Mixed $\alpha/\beta^{2,2}$-Peptides by Site-Selective Ring-Opening of Cyclic Quaternary Sulfamidates

Nuria Mazo,[‡] Iván García-González,[‡] Claudio D. Navo, Francisco Corzana, Gonzalo Jiménez-Osés, Alberto Avenoza, Jesús H. Busto,* and Jesús M. Peregrina*

Departamento de Química, Centro de Investigación en Síntesis Química. Universidad de La Rioja, C/Madre de Dios, 53, 26006 Logroño, La Rioja, Spain

Supporting Information

ABSTRACT: A method for site- and stereoselective peptide modification using a cyclic sulfamidate scaffold containing peptides is described. A peptide synthesis strategy allowing the rapid generation of mixed α/β-peptides incorporating a sulfamidate residue, derived from 2-methylisoserine, has been generalized. The unique electrophilic nature of this scaffold for nucleophilic substitution at a quaternary center with total inversion of its configuration, which was demonstrated computationally, allows for site-selective conjugation with various nucleophiles, such as anomeric thiocarbohydrates and pyridines. This strategy provides rapid access to complex thioglyco-α/β-conjugates and charged α/β-peptides.



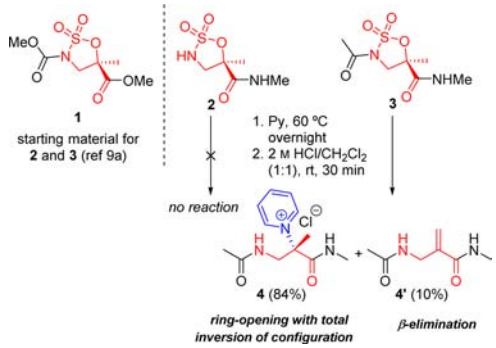
The synthesis of β-peptides is an emerging field with interesting and diverse applications, ranging from development of new functional materials to drug discovery.[1] The special properties of these peptides arise from the stabilization of unusual secondary structures providing a high degree of enzymatic stability.[2] More recently, the synthesis of hybrid α/β-peptides by combining proteinogenic α-amino acid residues and synthetic β-amino acids has contributed to extend the chemical space of available structures with potential applications. Therefore, various β-amino acids with different substitution patterns (β^2, β^3, $\beta^{2,3}$, and $\beta^{3,3}$) have been incorporated into peptides;[3] however, there are only a few cases reported for $\beta^{2,2}$-amino acid derivatives.[4] Among this type of amino acids, those incorporating electronegative groups at the α-position constitute a special case because they allow for modulating of the secondary structures of the peptides into which they are incorporated.[5]

Encouraged by well-established sulfamidate chemistry,[6] we have focused our interest on $\beta^{2,2}$-amino acids and developed a versatile synthetic methodology based on the ring-opening of electrophilic hindered cyclic sulfamidates with several oxygen, sulfur, or nitrogen nucleophiles.[7] We have demonstrated that this reaction proceeds with total inversion of the configuration of the quaternary electrophilic center, preserving the enantiomeric excess of the starting sulfamidate. One of the most attractive features of these sulfamidates is their ability to react with soft neutral nucleophiles such as pyridine, giving access to a new family of chiral, quaternary charged $\beta^{2,2}$-amino acids bearing a pyridinium substituent in the α-position.[8] These derivatives showed interesting conformational properties due to the rigidity imposed by the $N-CH_2-C-N^+$ dihedral angle.

In a previous work,[9] we reported that our parent cyclic sulfamidate **1** tolerates well the conversion of the ester group at the α-position to the peptide bond-mimicking methylamide group present in analogues **2** and **3**. These preliminary studies paved the way toward a more elaborate synthesis and site-selective modification of novel hybrid $\alpha/\beta^{2,2}$-peptides, which is presented herein.

We first evaluated the viability of our methodology using the minimal sulfamidate peptide models **2** and **3** as substrates and pyridine as nucleophile (Scheme 1). We found that (1) the presence of the *N*-acetyl group at the sulfamidate was critical for the ring-opening to proceed, (2) prolonged heating was necessary to achieve high substrate conversions at reasonable rates, and (3) small amounts of β-elimination reactions byproduct **4′** were obtained. Thus, heating acylated sulfamidate

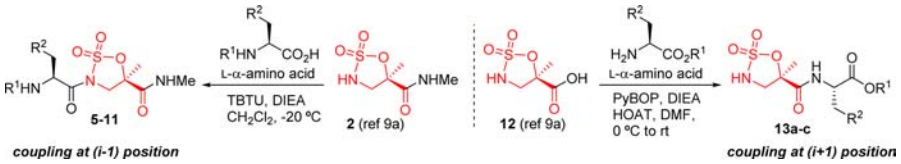
Scheme 1. Ring-Opening Reaction of Sulfamidates with Pyridine



Received: October 9, 2015
Published: November 16, 2015

DOI: 10.1021/acs.orglett.5b02927
Org. Lett. 2015, 17, 5804−5807

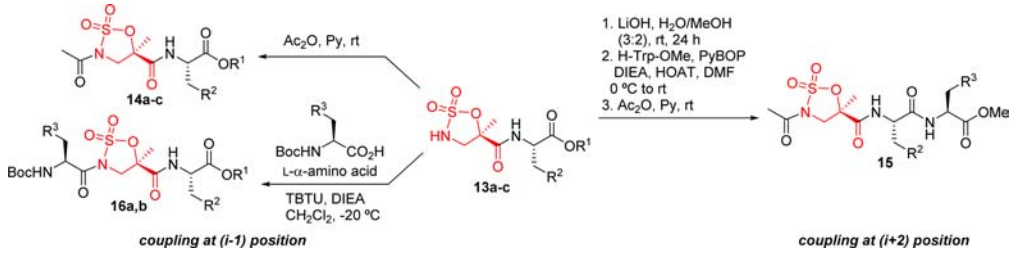
Table 1. Mixed $\alpha/\beta^{2,2}$-Dipeptides Incorporating a Cyclic Sulfamidate and α-Amino Acids at the (i−1) or (i+1) Positions



entry	R^1	R^2	Sul-α/β-peptide	yield (%)
1	Boc	H-	Boc-Ala-Sul-NHMe[a] (**5**)	82
2	Boc	Ph-	Boc-Phe-Sul-NHMe[a] (**6**)	79
3	Boc	(1*H*-indol-3-yl)-	Boc-Trp-Sul-NHMe[a] (**7**)	80
4	Boc	BnO-	Boc-Ser(OBn)-Sul-NHMe (**8**)	50
5	Fmoc	Ph-	Fmoc-Phe-Sul-NHMe (**9**)	73
6	Fmoc	Ph_3CS-	Fmoc-Cys(STr)-Sul-NHMe (**10**)	90
7	Boc	BnS-	Boc-Cys(SBn)-Sul-NHMe (**11**)	60
8	Me	H-	H-Sul-Ala-OMe (**13a**)	71
9	Bn	Ph-	H-Sul-Phe-OBn (**13b**)	87
10	Me	(1*H*-indol-3-yl)-	H-Sul-Trp-OMe (**13c**)	75

[a]Peptides described in ref 9a.

Table 2. Mixed α/β-Di- and Tripeptides Incorporating a Cyclic Sulfamidate and α-Amino Acids at the (i+1), (i+2), and (i−1) Positions



entry	R^1	R^2	R^3	Sul-α/β-peptide	yield (%)
1	Me	H-		Ac-Sul-Ala-OMe (**14a**)	59
2	Bn	Ph-		Ac-Sul-Phe-OBn (**14b**)	92
3	Me	(1*H*-indol-3-yl)-		Ac-Sul-Trp-OMe (**14c**)	81
4		H-	(1*H*-indol-3-yl)-	Ac-Sul-Ala-Trp-OMe (**15**)	34
5	Bn	Ph-	Ph-	Boc-Phe-Sul-Phe-OBn (**16a**)	87
6	Bn	Ph-	(1*H*-indol-3-yl)-	Boc-Trp-Sul-Phe-OBn (**16b**)	81

3 in pyridine at 60 °C overnight, followed by acidic workup, led to compound **4**, which can be regarded as the simplest α-pyridinium-substituted β-peptide model, in good yield (84%).

Our finding that acylation (or carbamoylation) of the sulfamic nitrogen triggers the nucleophilic ring-opening of otherwise unreactive quaternary sulfamidate has significant advantages for peptide synthesis: on one hand, the cyclic $NHSO_3$ moiety effectively protects the amino group of the 2-methylisoserine scaffold, obviating the typical *N*-protection/deprotection steps necessary for peptide coupling. On the other hand, it makes our strategy easily transferable to the site-selective modification of nonterminal peptides; however, for *N*-terminal peptides, a previous *N*-acylation step will be necessary prior to ring-opening.

As an extension of our preliminary studies,[9a] a small library of mixed $\alpha/\beta^{2,2}$-dipeptide derivatives was synthesized by coupling *N*-unprotected sulfamidate **2** to α-amino acids at the i−1 position in the presence of TBTU and DIEA in dichloromethane at −20 °C. Temperature control is crucial to avoid the ring-opening reaction with concomitant 1-hydroxybenzotriazole (HOBt) generated in the coupling reaction. These conditions allowed peptide coupling with the two most extensively used *N*-protecting groups (Boc in entries 1−4 and 7 and Fmoc in entries 5 and 6 of Table 1). Both nonpolar (Ala, Phe, Trp) and polar (Ser, Cys) amino acids were successfully coupled.

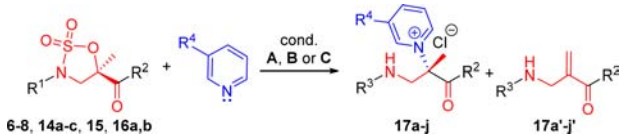
The coupling of α-amino acids at the i+1 position of the sulfamidate scaffold was achieved starting from unprotected sulfamidate **12**[10] using PyBOP and HOAT in the presence of DIEA as a base in DMF to obtain the mixed $\alpha/\beta^{2,2}$-dipeptides **13a−c** in good yields (entries 8−10, Table 1).

Once the ability to elongate the peptide chain in both directions was demonstrated, our library of mixed $\alpha/\beta^{2,2}$-peptides was expanded by either acetylating (dipeptides **14a−c**, entries 1−3 of Table 2) or coupling additional α-amino acids (tripeptides **16a,b**, entries 5 and 6 of Table 2) at the *N*-terminus of peptidic sulfamidates **13a−c**, in order to promote the subsequent ring-opening reaction. Additionally, tripeptide **15** was obtained from **13a** by basic hydrolysis of its methyl ester with LiOH, followed by coupling of H-Trp-OMe at the i+2 position and subsequent acetylation at the *N*-terminus (entry 4, Table 2).

The nucleophilic ring-opening reaction of several of these α/β-dipeptidic sulfamidates was successfully accomplished using pyridine derivatives under the conditions described above in Scheme 1 (Table 3). When starting from sulfamidates

DOI: 10.1021/acs.orglett.5b02927
Org. Lett. 2015, 17, 5804−5807

Table 3. Synthesis of Charged $\alpha/\beta^{2,2}$-Peptides[a] by Nucleophilic Ring Opening of Peptidic Sulfamidates with Pyridine



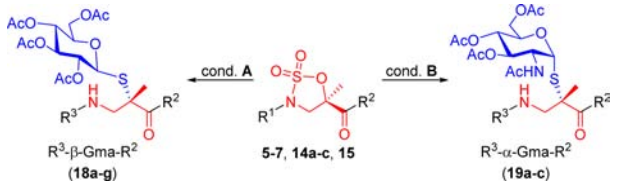
entry	Sul-α/β-peptide	cond[a]	α/β-peptide[b]	yield[c] (%)
1	**6**	A	Ac-Phe-Mpa-NHMe (**17a**)	62
2	**7**	A	Ac-Trp-Mpa-NHMe (**17b**)[d]	51
3	**8**	A	Ac-Ser(OBn)-Mpa-NHMe (**17c**)[d]	45
4	**8**	B	Ac-Ser(OBn)-Mpa'-NHMe (**17d**)	49
5	**16a**	A	Ac-Phe-Mpa-Phe-OBn (**17e**)[d]	30
6	**16b**	A	Ac-Trp-Mpa-Phe-OBn (**17f**)[d]	33
7	**14a**	C	Ac-Mpa-Ala-OMe (**17g**)[d]	60
8	**14b**	C	Ac-Mpa-Phe-OBn (**17h**)[d]	70
9	**14c**	C	Ac-Mpa-Trp-OMe (**17i**)[d]	52
10	**15**	C	Ac-Mpa-Ala-Trp-OMe (**17j**)[d]	53

[a]Conditions **A**, **B**: (1) pyridine (cond **A**) or 3-methylpyridine (cond **B**), 60 °C, overnight; (2) aq 2 M HCl/CH_2Cl_2 (1:1), rt, 30 min; (3) TFA/CH_2Cl_2 (1:2), rt, 30 min; (4) Ac_2O/Py (1:2), rt, 1 h. Conditions **C**: (1) pyridine, 60 °C, overnight; (2) aq 2 M HCl/CH_2Cl_2 (1:1), rt, 30 min. [b]Mpa = 2-methyl-2-(pyridinium)-3-amino acid. Mpa′ = 2-methyl-2-(3′-methylpyridinium)-3-amino acid. [c]Yield is referred to the compound obtained after purification by column chromatography or semipreparative HPLC affording in some cases low yields because the separation was difficult. [d]Small amounts (<17%) of the corresponding elimination byproducts (dehydropeptides, **17a′−j′**) were observed (see the Supporting Information).

possessing α-amino acids at the $i-1$ position (**6-8** and **16a,b**), charged $\alpha/\beta^{2,2}$-dipeptides **17a−c** (entries 1−3, Table 3) and $\alpha/\beta^{2,2}$-tripeptides **17e,f** (entries 5 and 6, Table 3) were obtained after removal of the Boc group with TFA and acetylation of the *N*-terminus, albeit in moderate yields after exhaustive purification. Peptidic sulfamidate **8** was ring-opened with bulkier 3-methylpyridine under the same conditions to give $\alpha/\beta^{2,2}$-peptide **17d** with similar yield (entry 4, Table 3). Analogously, $\alpha/\beta^{2,2}$-dipeptides (**17g−i**) and a $\alpha/\beta^{2,2}$-tripeptide (**17j**) incorporating α-amino acids at the $i+1$ and ($i+1$, $i+2$) positions were also prepared in moderate to good yields (entries 7−9 and 10 of Table 3, respectively). In all cases, the opening reaction proceeded with total inversion of the configuration at the quaternary center, and in most of the reactions, small amounts of elimination byproducts (dehydro-α/β-peptides **17a−j′**) were observed.

In order to generalize this methodology for the synthesis of other important hybrid conjugates, we attempted the ring-opening reaction of these peptidic sulfamidates with 1-thiocarbohydrate derivatives. Thus, treatment of sulfamidates **5−7**, **14a−c**, and **15** with tetra-*O*-acetyl-β-1-thio-D-glucopyranose using DBU as a base in DMF at room temperature, followed by acid hydrolysis of the sulfamic and Boc moieties and further acetylation of the *N*-terminus, led to *S*-glyco-α/β-dipeptides **18a−g** in which a β-D-glucopyranose is linked to a $\beta^{2,2}$-amino acid through a *S*-glycosidic bond in good yields (entries 1−7, Table 4). The analogous treatment of sulfamidates **14a,c** and **15** with tri-*O*-acetyl-α-1-thio-*N*-acetylglucosamine yielded *S*-glyco-α/β-peptides **19a−c** bearing a α-thioglycosidic bond in good yields (entries 8−10, Table 4).

Table 4. Synthesis of *S*-Glyco-$\alpha/\beta^{2,2}$-peptides by Nucleophilic Ring Opening of Peptidic Sulfamidates with 1-Thiocarbohydrate Derivatives[a]



entry	Sul-α/β-peptide	*S*-glyco-α/β-peptide[b]	yield (%)
1	**5**	Ac-Ala-β-Gma-NHMe (**18a**)[c]	37
2	**6**	Ac-Phe-β-Gma-NHMe (**18b**)[c]	79
3	**7**	Ac-Trp-β-Gma-NHMe (**18c**)[c]	80
4	**14a**	Ac-β-Gma-Ala-OMe (**18d**)	74
5	**14b**	Ac-β-Gma-Phe-OBn (**18e**)	65
6	**14c**	Ac-β-Gma-Trp-OMe[d] (**18f**)	66[d]
7	**15**	Ac-β-Gma-Ala-Trp-OMe[d] (**18g**)	76[d]
8	**14a**	Ac-α-Gma-Ala-OMe (**19a**)	68
9	**14c**	Ac-α-Gma-Trp-OMe (**19b**)	61
10	**15**	Ac-α-Gma-Ala-Trp-OMe[d] (**19c**)	67[d]

[a]Conditions **A**, **B**: (1) $(Ac)_4$Glc-β-SH (conditions **A**) or $(Ac)_3$GlcNAc-α-SH (conditions **B**), DBU, DMF, rt; (2) H_2SO_4/CH_2Cl_2 (1:1); (3) TFA/CH_2Cl_2 (1:2), rt, 30 min; (4) Ac_2O/Py (1:2), rt, 1 h. [b]β- or α-Gma = 2-(β- or α-*S*-glycosyl)-2-methyl-3-amino acid. [c]See ref 9a. [d]For an adequate purification by semipreparative HPLC, it was necessary to deprotect the glycan hydroxyl group with NaOMe/MeOH at rt for 1 h.

Ring-opening reactions proceeded with complete inversion of the configuration in all cases, and competitive elimination reactions were never observed.

The conformational preferences of hybrid peptides **17a** and **17b** in water were investigated by molecular dynamics (MD) simulations and corroborated by NOE experiments. While the natural residue (Phe or Trp) adopts a distribution of extended and folded conformations in both compounds, the $\beta^{2,2}$-amino acid is quite rigid, with θ and ψ values around 180°. The value of θ dihedral angle differs from that observed in glycopeptides **18b** and **18c**, with values close to 60°.[9a] The spatial disposition of the backbone of peptides **17a** and **17b** allows persistent π-stacking interactions between the aromatic side chains (phenyl/indolyl and pyridinium moieties), which is present about 25% of the total simulation time and is validated by NOE cross-peaks between aromatic protons (Figure 1 and the Supporting Information).

In summary, we synthesized a small library of 37 hybrid $\alpha/\beta^{2,2}$-peptides, 16 of them incorporating a protected $\beta^{2,2}$-amino

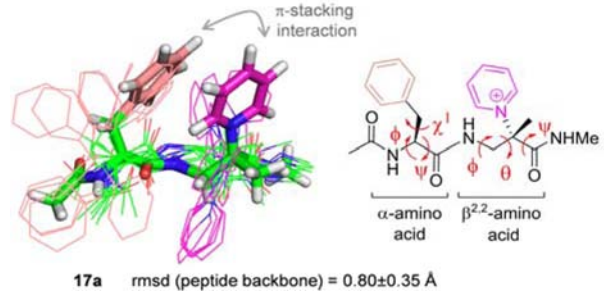


Figure 1. Calculated ensembles for peptide **17a** obtained from 100 ns MD simulations (rmsd = root-mean-square deviation).

DOI: 10.1021/acs.orglett.5b02927
Org. Lett. 2015, 17, 5804−5807

acid as a cyclic sulfamidate, and simultaneously activated for subsequent nucleophilic ring-opening. These activated peptides were able to undergo site-selective modification through nucleophilic opening reactions with total inversion of the configuration at the quaternary α-carbon. From these reactants, 10 charged hybrid α/β-peptides bearing the pyridinium moiety, which has been reported to confer special conformational properties to the peptide backbone, were prepared. Additionally, we synthesized 10 *S*-glycosylated hybrid α/β-peptides through the late-stage stereoselective nucleophilic opening of peptidic sulfamidates with 1-thiocarbohydrates derived from β-D-glucopyranose and α-D-*N*-acetylglucosamine. Taking into account the emergence of *S*-glycosylation, this synthetic strategy allows both α- and β-*S*-glycosylation of peptides in a chemoselective and, more importantly, stereocontrolled way. The combination of α- and $\beta^{2,2}$-amino acids in designed hybrid α/β-peptides and *S*-glycosylated α/β-glycopeptides offers new opportunities to access and stabilize novel architectures not previously observed in α- or β-peptides with potential applications in molecular recognition and drug discovery.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02927.

Experimental and computational details as well as copies of NMR spectra for all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: hector.busto@unirioja.es.
*E-mail: jesusmanuel.peregrina@unirioja.es.

Author Contributions

‡N.M. and I.G.-G. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the Ministerio de Economía y Competitividad (projects CTQ2012-36365/BQU and UNLR13-4E-1931, Ramon y Cajal contract of G.J.O.) and the Gobierno de La Rioja (predoctoral fellowship of I.G.-G.). We also thank CESGA and BiFi (Memento cluster) for computational support.

■ REFERENCES

(1) (a) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, *1*, 1111. (b) Johnson, L. M.; Gellman, S. H. *Methods Enzymol.* **2013**, *523*, 407. (c) Cabrele, C.; Martinek, T. A.; Reiser, O.; Berlicki, L. *J. Med. Chem.* **2014**, *57*, 9718. (d) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399.

(2) (a) Cardillo, G.; Gentilucci, L.; Qasem, A. R.; Sgarzi, F.; Spaminato, S. *J. Med. Chem.* **2002**, *45*, 2571. (b) Hook, D. F.; Bindschadler, P.; Mahajan, Y. R.; Sebesta, R.; Kast, P.; Seebach, D. *Chem. Biodiversity* **2005**, *2*, 591. (c) Soudy, R.; Gill, A.; Sprules, T.; Lavasanifar, A.; Kaur, K. *J. Med. Chem.* **2011**, *54*, 7523. (d) De Marco, R.; Mazzotti, G.; Dattoli, S. D.; Baiula, M.; Spampinato, S.; Greco, A.; Gentilucci, L. *Biopolymers* **2015**, *104*, 636. (e) Greco, A.; Tani, S.; De Marco, R.; Gentilucci, L. *Chem. - Eur. J.* **2014**, *20*, 13390.

(3) (a) Pilsl, L. K. A.; Reiser, O. *Amino Acids* **2011**, *41*, 709. (b) Vasudev, P. G.; Chatterjee, S.; Shamala, N.; Balaram, P. *Chem. Rev.* **2011**, *111*, 657. (c) Martinek, T. A.; Fülöp, F. *Chem. Soc. Rev.* **2012**, *41*, 687. (d) Johnson, L. M.; Barrick, S.; Hager, M. V.; McFedries, A.; Homan, E. A.; Rabaglia, M. E.; Keller, M. P.; Attie, A. D.; Saghatelian, A.; Bisello, A.; Gellman, S. H. *J. Am. Chem. Soc.* **2014**, *136*, 12848. (e) Checco, J. W.; Lee, E. F.; Evangelista, M.; Sleebs, N. J.; Rogers, K.; Pettikiriarachchi, A.; Kershaw, N. J.; Eddinger, G. A.; Belair, D. G.; Wilson, J. L.; Eller, C. H.; Raines, R. T.; Murphy, W. L.; Smith, B. J.; Gellman, S. H.; Fairlie, D. *J. Am. Chem. Soc.* **2015**, *137*, 11365.

(4) See, for example: (a) Lengyel, G. A.; Horne, W. S. *J. Am. Chem. Soc.* **2012**, *134*, 15906. (b) Grellepois, F. *J. Org. Chem.* **2013**, *78*, 1127. (c) Basuroy, K.; Karuppiah, V.; Balaram, P. *Org. Lett.* **2014**, *16*, 4614.

(5) Rodríguez, F.; Corzana, F.; Busto, J. H.; Avenoza, A.; Peregrina, J. M.; Zurbano, M. M. *Curr. Top. Med. Chem.* **2014**, *14*, 1225.

(6) (a) Bower, J. F.; Švenda, J.; Williams, A. J.; Charmant, J. P. H.; Lawrence, R. M.; Szeto, P.; Gallagher, T. *Org. Lett.* **2004**, *6*, 4727. (b) Cohen, S. B.; Halcomb, R. L. *J. Am. Chem. Soc.* **2002**, *124*, 2534. (c) Jamieson, A. G.; Boutard, N.; Beauregard, K.; Bodas, M. S.; Ong, H.; Quiniou, C.; Chemtob, S.; Lubell, W. D. *J. Am. Chem. Soc.* **2009**, *131*, 7917. (d) Kim, J.; Seo, Y. J.; Kang, S.; Han, J.; Lee, H.-K. *Chem. Commun.* **2014**, *50*, 13706.

(7) (a) Avenoza, A.; Busto, J. H.; Corzana, F.; Jiménez-Osés, G.; Peregrina, J. M. *Chem. Commun.* **2004**, 980. (b) Avenoza, A.; Busto, J. H.; Jiménez-Osés, G.; Peregrina, J. M. *J. Org. Chem.* **2006**, *71*, 1692. (c) Avenoza, A.; Busto, J. H.; Jiménez-Osés, G.; Peregrina, J. M. *Org. Lett.* **2006**, *8*, 2855. (d) Avenoza, A.; Busto, J. H.; Jiménez-Osés, G.; Peregrina, J. M. *J. Org. Chem.* **2005**, *70*, 5721. (e) Jiménez-Osés, G.; Avenoza, A.; Busto, J. H.; Rodríguez, F.; Peregrina, J. M. *Chem. - Eur. J.* **2009**, *15*, 9810. (f) Mata, L.; Jiménez-Osés, G.; Avenoza, A.; Busto, J. H.; Peregrina, J. M. *J. Org. Chem.* **2011**, *76*, 4034.

(8) Mata, L.; Avenoza, A.; Busto, J. H.; Corzana, F.; Peregrina, J. M. *Chem. - Eur. J.* **2012**, *18*, 15822.

(9) (a) García-González, I.; Mata, L.; Corzana, F.; Jiménez-Osés, G.; Avenoza, A.; Busto, J. H.; Peregrina, J. M. *Chem. - Eur. J.* **2015**, *21*, 1156. (b) Mata, L.; Avenoza, A.; Busto, J. H.; Peregrina, J. M. *Chem. - Eur. J.* **2013**, *19*, 6831.

(10) Jiménez- Osés, G.; Avenoza, A.; Busto, J. H.; Peregrina, J. M. *Tetrahedron: Asymmetry* **2008**, *19*, 443.

DOI: 10.1021/acs.orglett.5b02927
Org. Lett. 2015, 17, 5804−5807