JOC The Journal of Organic Chemistry
Cite This: J. Org. Chem. 2019, 84, 8392−8410
Article
pubs.acs.org/joc

Conversion of "Customizable Units" into *N*-Alkyl Amino Acids and Generation of *N*-Alkyl Peptides

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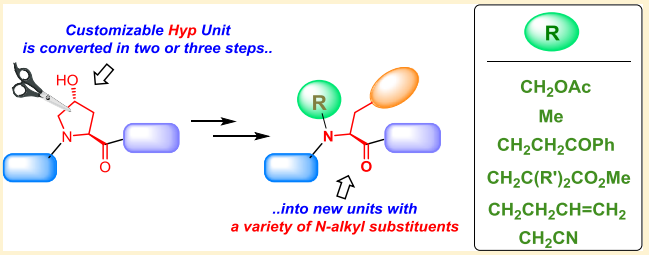
Supporting Information

ABSTRACT: An efficient conversion of hydroxyproline "customizable" units into new amino acids with a variety of *N*-alkyl substituents is described. The process is versatile and can afford valuable *N*-methyl amino acids and *N*,*O*-acetals. In addition, it allows the introduction of *N*-homoallylic substituents and N-chains with terminal ester, ketone, or cyano groups. These chains could be used for peptide extension or conjugation to other molecules (e.g., by olefin metathesis, peptide ligation, etc.). The transformation is carried out in just two (for R = CH_2OAc) or three steps (scission of the pyrrolidine ring, manipulation of the α-chain, and the *N*-substituent) under mild, metal-free conditions, affording products with high optical purity.



INTRODUCTION

N-Alkyl amino acids are valuable building blocks[1] because their incorporation into peptides can modify their conformation, by decreasing the number of hydrogen bonds and increasing backbone steric hindrance,[2] and therefore, modulate their biological properties,[3] such as potency, selectivity, and bioavailability.[4] Thus, *N*-alkyl amino acids increase peptide resistance to proteases[1,5] and their cell permeability.[1,6]

A variety of *N*-methyl amino acids can be found in natural products,[7] such as the insecticide cycloaspeptide E (Figure 1),[8] the antimicrobials enniatin[9] and ilamycins,[10] the immunosuppressant cyclosporine,[11] and the antitumorals efrapeptins and dolastatins.[12] Moreover, the generation of *N*-methylated analogues of natural products by Kessler and others[1e] has led to improved pharmacological profiles, for instance, allowing oral bioavailability for the growth hormone somatostatin and improving its potency and selectivity.[13] In another example, the *N*-methyl scan for the cyclic peptide c(RGDfV), which is selective for α_v integrins, resulted in the potent antitumoral cilengitide (Figure 1), which reached phase III clinical trials before being granted orphan drug status for glioma.[14]

Although much less frequent than the *N*-methyl group, other *N*-alkyl units can be found in amino acids and peptides, such as the acyloxymethyl group in antimitotic peptide tubulysin (Figure 1)[15] or ethyl, propyl, butyl, and benzyl groups, among others.[1,16] Recently, the ethylene glycol group was used in lipophilic analogues of antitumoral sansalvamide.[16a] Other alkyl chains have been attached to the amide group at the *C*- or *N*-terminal positions.[14,15] Thus, the *N*-ethylation of the *C*-terminal position of the luteinizing hormone-releasing hormone afforded analogues with increased potency.[17] The introduction of alkyl chains into the *N*-terminus of an ETAV derivative yielded stable, cell-permeable inhibitors of the interaction between the *N*-methyl-D-aspartate receptor and PSD95.[18]

The nature of the alkyl group is important for the bioactivity. Thus, if the *N*-methyl leucine of cyclosporine is replaced by *N*-ethyl amino acids, the immunosuppressive activity is lost, but an anti-HIV agent is obtained instead.[19] In a different example, if the *N*,*O*-acetal of tubulysin D (Figure 1) is replaced by a *N*-Me

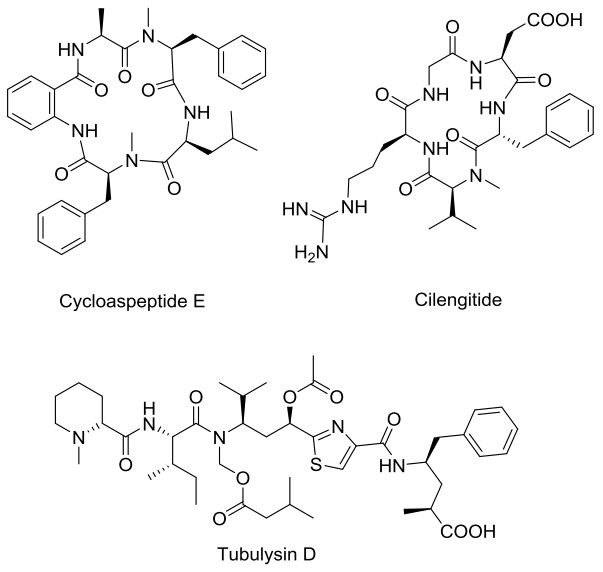


Figure 1. *N*-Alkyl groups in bioactive natural products and analogues.

Received: January 15, 2019
Published: May 23, 2019

DOI: 10.1021/acs.joc.9b00114
J. Org. Chem. 2019, 84, 8392−8410

moiety, a considerable increase in stability was achieved, although a two-fold drop in activity was observed as well.[15]

Because of their importance, the commercial availability of *N*-methyl amino acids has increased in recent years, but they are still expensive. Moreover, other *N*-alkyl amino acids are scarce.

Several methods have been developed to prepare *N*-methyl amino acids,[1,20−23] in order to overcome problems such as epimerization of the intermediates, cleavage of protecting groups, overalkylation, undesired methylation of the lateral chain groups, long reaction times, and expensive or dangerous reagents. Thus, in the initial reductive amination methods, imines were generated and then reduced by hydrogen or sodium borohydride.[21] A modification developed by Freidinger generated an oxazolidinone which was reduced with triethylsilane in acid media, a procedure compatible with Fmoc protecting groups.[22]

In other approaches, the *N*-protected amino acid derivative was treated with an alkylating agent,[23] such as methyl iodide, diazomethane, and trimethyloxonium tetrafluoroborate. A variant uses the Mitsunobu conditions (methanol, DIAD, PPh_3). An important advance was achieved by Fukuyama, Miller, and Scanlan, with the introduction of the nosyl group to activate the desired position because this group can be cleaved afterward under relatively mild conditions.[24] Finally, some sustainable, catalytic methodologies have recently been reported.[21a,d,25]

For the synthesis of other *N*-alkyl amino acids with bulkier alkyl chains, a few of these methodologies (such as reductive amination and alkylation of *N*-nosyl derivatives) have been adapted. However, the availability of these amino acids is still quite limited.

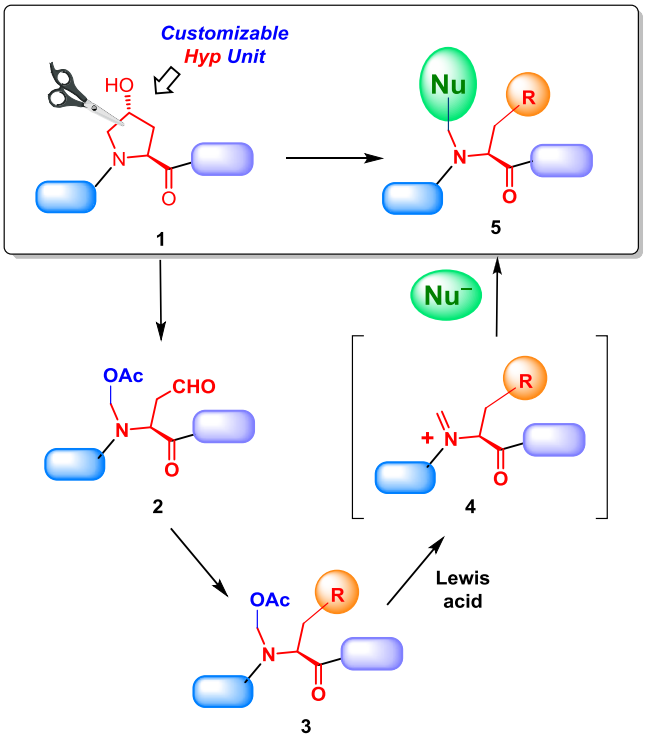
Once the *N*-alkyl amino acid is available, a second problem arises: its incorporation into peptides. Both in conventional liquid- or solid-phase synthesis,[1,26] and using biosynthetic systems,[27] the incorporation of *N*-alkyl amino acids is tricky.[1,26−28] Slow coupling rates, side-reactions such as racemization, double hit incorporation, deletion, and also peptide fragmentation and diketopiperazine formation are common problems found during these couplings.[1a] In spite of the development of new coupling reagents and microwave activation,[26] the coupling step remains challenging. The bulkier the *N*-alkyl group is, the more side-reactions are likely to occur.

Therefore, a method which could provide a variety of *N*-alkyl amino acids from a low-cost substrate without epimerization, overalkylation, or expensive reagents would be quite interesting. If this methodology could be adapted to the site-selective generation of *N*-alkyl amino acids into peptides, even when very bulky chains are involved, the procedure would have a wide range of applications in synthetic and medicinal chemistry.

In a previous report, we described the scission of natural, inexpensive hydroxyproline units **1** (Scheme 1) to give *N*-acetoxymethyl-4-oxohomoalanines **2** and the manipulation of the α-chain (conversion **2** → **3**).[29] In this article, we address an additional challenge: the introduction of different *N*-alkyl substituents (conversion **3** → **5**) to afford a diversity of *N*-alkyl amino acids **5** with high optical purity.

In addition, we study the formation of *N*-alkyl amino acids in small peptides by site-selective modification of Hyp units. While the traditional coupling is particularly troublesome for residues with bulky *N*-substituents, the introduction of Hyp units into peptides proceeds readily. The five-membered ring poses less steric hindrance than a freely rotating alkyl group; in fact, proline and Hyp are the only *N*-alkyl amino acids in the genetic code due to their superior reactivity.[1,30] Once Hyp is incorporated in a certain position, cleavage of the ring under mild conditions will unfold a new amino acid with a customizable *N*-substituent.[31,32]

Scheme 1. Conversion of Customizable Hyp Units into β-Substituted Dehydroamino Acids



The feasibility of this approach to give a diversity of alkyl chains will be described herein.

RESULTS AND DISCUSSION

In order to study the scission of hydroxyproline units and the manipulation of the α-alkyl chain, *N*-benzoyl hydroxyproline **6** (Table 1) and dipeptides **7**[29b] and **8** were prepared and used as scission substrates. The scission was carried out under usual conditions,[29] with (diacetoxyiodo)benzene and iodine under visible light irradiation at reflux, affording the scission products **9**−**11** in good yields.[33]

Then, the α-side chain was manipulated by reductive amination because we were particularly interested in the production of cationic amino acids for antimicrobial peptide synthesis and in the production of branched peptides for new materials. The reductive amination proceeded in good to excellent yields to give products **12**−**15**.

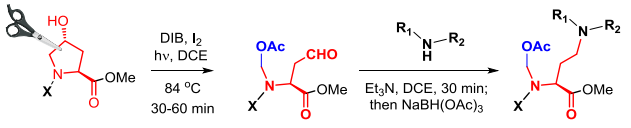
The reduction of the *N*,*O*-acetal to a *N*-methyl group was studied with amino acid **12** and dipeptide **14** (Scheme 2). Under treatment with a Lewis acid, an acyliminium ion was generated that reacted with the hydride source Et_3SiH, affording *N*-methyl derivatives **16** and **17** in very good global yield.

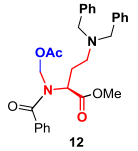
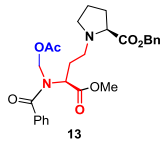
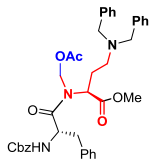
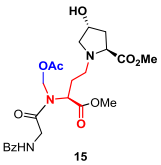
The generation of amino acids and peptides presenting bulky *N*-alkyl groups was studied under different conditions (Scheme 3 and Table 2), using as substrates the *N*,*O*-acetals **12**−**15**. In all cases, the reaction mixture was cooled to 0 °C before adding the Lewis acid. The resulting iminium intermediates were trapped by dropwise addition of the *C*-nucleophile, acetophenone (trimethyl)silyl enol ether, to afford *N*-alkylated products **18**−**21**.

As seen in Table 2, entrances 1−3, the best conditions for the conversion of substrate **12** into the *N*-alkylated product **18** (entry 3) used TMSOTf as the Lewis acid and MeCN as the solvent. To our satisfaction, the introduction of the bulky *N*-substituent took place in excellent yield. Replacement of

DOI: 10.1021/acs.joc.9b00114
J. Org. Chem. 2019, 84, 8392−8410

Table 1. Conversion of Customizable Hyp Units into Compounds 12−15



Entry	Scission substrate	Scission product (%)	Amine for the reductive amination	Reductive amination product[c]	Yield (%)[d]
1	**6**	**9** (80%)[a]	Ph_2NH	 **12**	92
2	**6**	**9** (80%)[a]	**Pro-OBn**	 **13**	78
3	**7**	**10** (82%)[b]	Ph_2NH	 **14**	84
4	**8**	**11** (71%)[b]	**Hyp-OMe**	 **15**	85

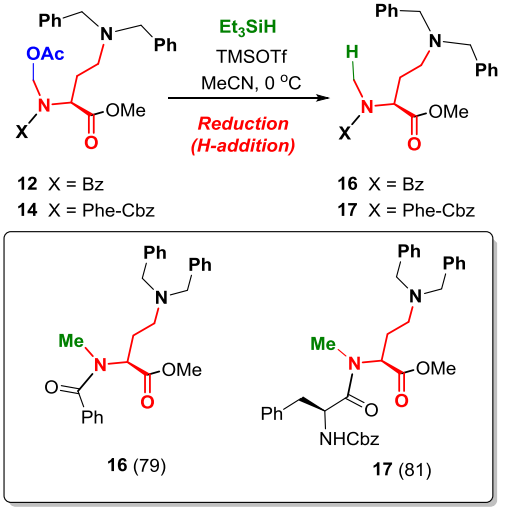
[a]Scission conditions: substrate (0.2 mmol), DIB (0.4 mmol), I_2 (0.1 mmol), DCE (4 mL), 84 °C, 30 min. [b]Scission conditions as in (a) but using more iodine (0.2 mmol) and reaction time (60 min). [c]Reductive amination conditions: substrate (0.15 mmol), amine (0.17 mmol), Et_3N (0.2 mmol), 30 min; then, $NaBH(OAc)_3$ (0.45 mmol). [d]Yields after purification by chromatography on silica gel.

MeCN by dichloromethane (entries 2 and 3) or replacement of TMSOTf by BF_3OEt_2 (entries 1 and 2) resulted in low to moderate yields. The optimized conditions were tried with dipeptides **13** (entry 4) and **14** (entry 5), affording products **19** and **20** in good yields (65 and 87%, respectively). Even the branched tripeptide **15** underwent the reaction to give product **21** in 84% yield (entry 6). Remarkably, no epimers of products **18**−**21** could be isolated.

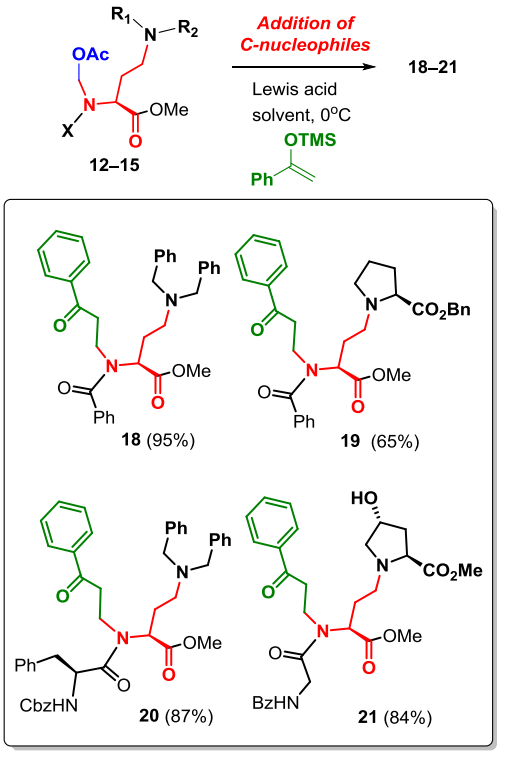
The optimized conditions were then applied to the addition of other *C*-nucleophiles (Scheme 4). Using amino acid **12** and dipeptide **14** as substrates, the addition of a silyl ketene gave products **22** and **23** in good yields, in spite of the bulkiness of the *N*-alkyl chain. Silyl ketones can be easily prepared, and a variety of chains with *α*-substituted esters can be introduced. The presence of a terminal carboxyl group is interesting because it can be used to attach other peptide chains or conjugate other molecules.

The addition of TMSCN also proceeded in good yields to afford cyanides **24** and **25**. The CN groups can undergo many transformations to extend the lateral chain, which we are currently exploring. Finally, the addition of allylTMS yields homoallylic chains (in compounds **26** and **27**), which can be used to extend the lateral chain using olefin metathesis, increasing the diversity of the *N*-alkyl chains.

Scheme 2. Reduction of *N,O*-Acetal To Give *N*-Methyl Derivatives



Scheme 3. Optimization of the Introduction of Bulky *N*-Alkyl Chains (See Also Table 2)



In the NMR spectra at 26 °C of many carbamates and amides, broad bands were observed because of overlapping of the signals of several rotamers. In those cases, because heating of the samples can increase rotamer interconversion, the spectra at 70 °C were recorded.[34] In the case of compound **25**, even at 70 °C, some minor rotamers could be observed; only at 100 °C, the signals coalesced (Supporting Information).

The *α*-chain of the scission products can also undergo other transformations. In the example shown in Scheme 5, *tert*-butyl

DOI: 10.1021/acs.joc.9b00114
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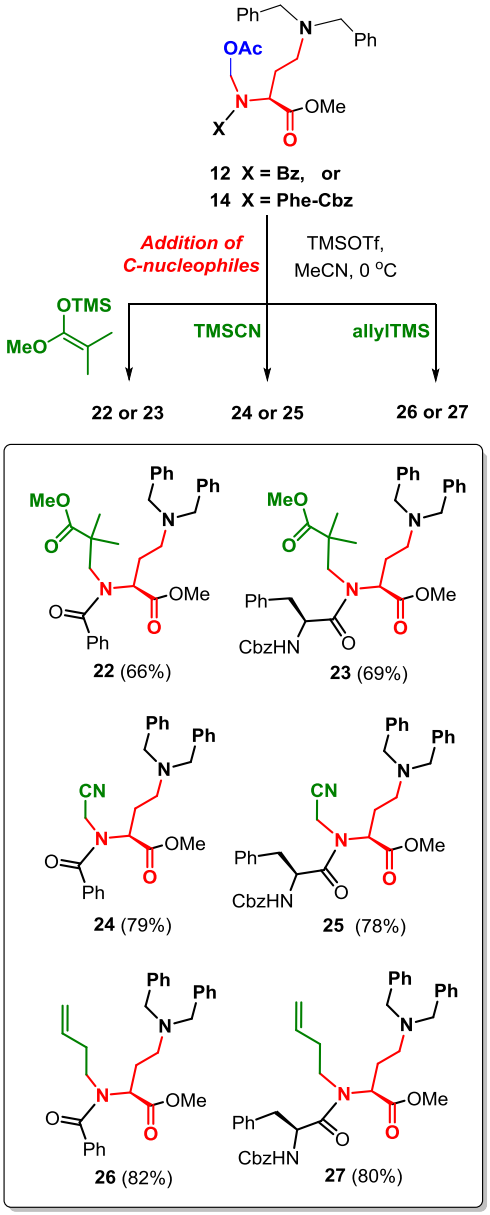
Table 2. Optimization of the Introduction of Bulky *N*-Alkyl Chains

entry	substrate	addition conditions	product (yield)[a]
1	12	**Ph−C(OTMS)=CH$_2$** (5 equiv), CH_2Cl_2, BF_3OEt_2 (2 equiv), 0 °C, 5 h	**18** (23%)
2	12	**Ph−C(OTMS)=CH$_2$** (5 equiv), CH_2Cl_2, TMSOTf (2 equiv), 0 °C, 5 h	**18** (40%)
3	12	**Ph−C(OTMS)=CH$_2$** (5 equiv), MeCN, TMSOTf (2 equiv), 0 °C, 5 h	**18** (95%)
4	13	**Ph−C(OTMS)=CH$_2$** (5 equiv), MeCN, TMSOTf (2 equiv), 0 °C, 5 h	**19** (65%)
5	14	**Ph−C(OTMS)=CH$_2$** (5 equiv), MeCN, TMSOTf (2 equiv), 0 °C, 5 h	**20** (87%)
6	15	**Ph−C(OTMS)=CH$_2$** (5 equiv), MeCN, TMSOTf (2 equiv), 0 °C, 5 h	**21** (84%)

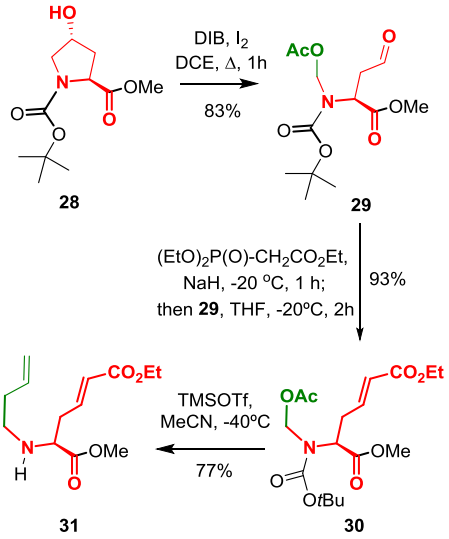
[a]Yields for products purified by chromatography.

Scheme 4. Conversion of the *N,O*-Acetal Group into Different *N*-Alkyl Chains



carbamate **28** underwent oxidative scission to afford aldehyde **29**. This scission product was treated under

Scheme 5. Transformation of the α-Chain Using HWE Reaction



Horner−Wadsworth−Emmons (HWE) conditions to give valuable dehydro homoglutamic acid derivative **30**, which was then treated with allyltrimethylsilane in the presence of TMSOTf. Remarkably, the resulting addition product also underwent cleavage of the Boc group, releasing free *N*-alkyl amino acid **31**. These unprotected amino acids are useful synthetic intermediates.

The manipulation of the α-chain of the scission products and the subsequent transformation of the *N,O*-acetals can provide many different products. In the example shown in Scheme 6, tripeptide **32** was transformed into scission product **33**, which was not isolated. The crude aldehyde underwent HWE reaction to afford derivative **34**, which was then treated with *C*-nucleophiles in the presence of TMSOTf.

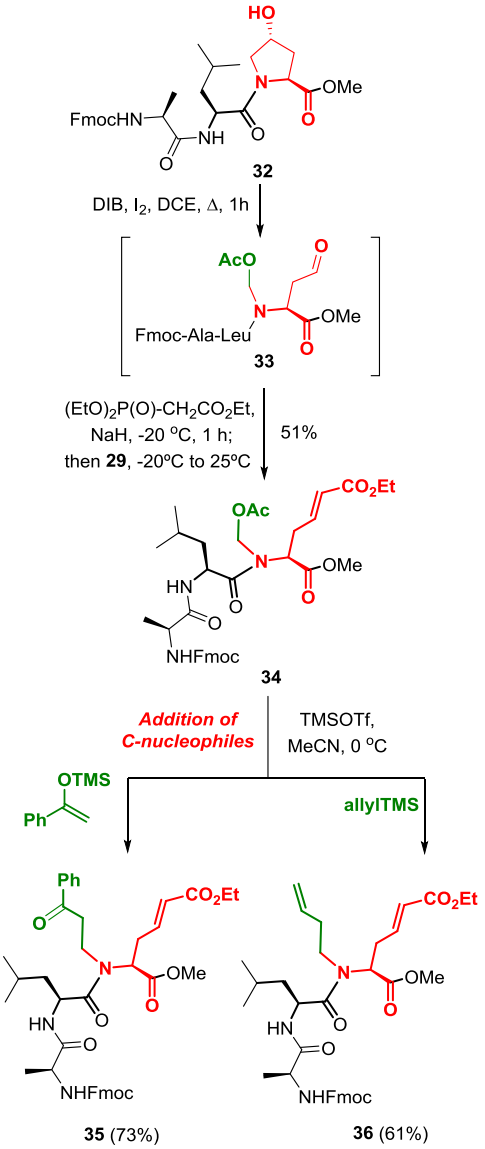
Thus, reaction with acetophenone trimethylsilyl ether afforded product **35** in 73% yield. The introduction of aromatic ketones can be used to generate fluorescent probes, and the reductive amination of the ketone could generate branched peptide derivatives.

On the other hand, treatment of *N,O*-acetal **34** with allylTMS gave diolefinic derivative **36**, whose lateral chains can be functionalized independently.

The presence of *N*-alkyl amino acids in peptides hinders rotamer interconversion, which can be useful for modulating the conformation and biological activity of the peptides.[35] The present procedure allows comparison of the conformational and biological properties of peptide series where a cyclic amino acid is replaced by a lineal *N*-alkylated amino acid, and then by a lineal, non *N*-alkylated residue. An example of the preparation of such series is shown in Scheme 7. The natural peptide goralatide Ac-Ser-Asp-Lys-Pro-OH is a regulator of hematopoiesis and inhibits the entry of murine and human hemaotopoietic stem cells into the S-phase.[36] Compound **37** was prepared as a precursor of hydroxylated goralatide. But in addition, the Hyp residue was fragmented, and the intermediate aldehyde (not shown) underwent a HWE reaction to give an *N*-alkyl residue in compound **38**. The effect of *N*-alkylation can be studied by removal of the *N*-acetoxymethyl group by hydrolysis (compound **39**). The terminal homoglutamic unit was introduced because a protected ester behaves as a neutral unit, but it can also be converted into an acidic unit if the ester is saponified, or a

DOI: 10.1021/acs.joc.9b00114
J. Org. Chem. 2019, 84, 8392−8410

Scheme 6. Library Diversification by α-Chain and *N*,*O*-Acetal Manipulations



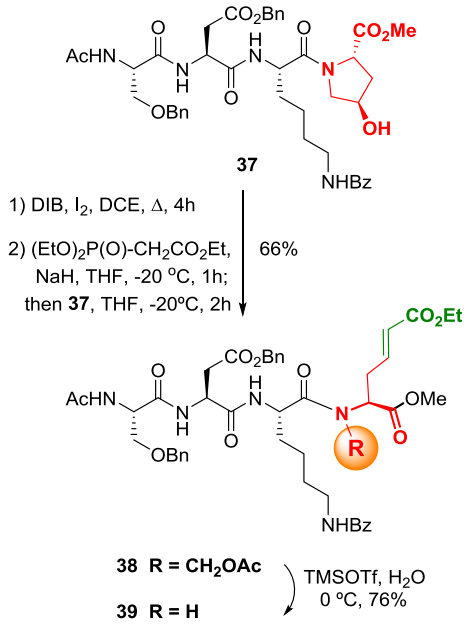
cationic unit if derivatized as an amide. The generation of a library of goralatide analogues and the study of their conformation and bioactivity are currently underway and will be reported in the future. Because the current methodology allows the formation of a variety of unalkylated and *N*-alkyl amino acids in peptides, it will be very useful to study conformational and pharmacological effects for bioactive peptides.

CONCLUSIONS

The efficient transformation of hydroxyproline "customizable" units into lineal amino acids with a variety of *N*-alkyl substituents is achieved. The conversion is carried out in three (or less) steps, including scission of the pyrrolidine ring and manipulation of the α-chain and the *N*-substituent under mild, metal-free conditions, yielding products with high optical purity.

Thus, the scission of the Hyp unit generates α-alkyl and *N*-acetoxymethyl chains which can be transformed independently. The conversion of the *N*-acetoxymethyl chain was carried out by reduction to give valuable *N*-methyl amino acid derivatives or by addition of *C*-nucleophiles to give *N*-homoallylic substituents

Scheme 7. Preparation of Rigid and Flexible Derivatives for Conformational and Bioactivity Studies by Replacement of Cyclic Residues (Hyp) by *N*-Alkyl Residues and *N*-Unalkylated Units



and *N*-chains with terminal ester, ketone, or cyano groups. These chains were introduced in good to excellent yields, even when quite bulky ones were generated. The chains presented functionalities that could be useful to further extend the peptide or to conjugate it with other molecules.

This methodology could be a valuable alternative to the conventional introduction of *N*-alkyl amino acids into peptides because the coupling of residues with bulky *N*-substituents can be troublesome. In contrast, introduction of Hyp units into peptides is straightforward, and their subsequent conversion into amino acids with bulky *N*-alkyl chains proceeds efficiently.

EXPERIMENTAL SECTION

General Remarks: General Methods. Commercially available reagents and solvents were of analytical grade or were purified by standard procedures prior to use. All reactions involving air- or moisture-sensitive materials were carried out under a nitrogen atmosphere. Potassium permanganate solution [obtained by dissolving 10 g $KMnO_4$, 66.7 g K_2CO_3, and 0.85 g NaOH in 1 L of water] is used as the thin-layer chromatography (TLC) stain. The TLC plate is plunged into the stain, and the TLC plate was heated until the color developed.

Merck silica gels 60 PF_{254} and 60 (0.063−0.2 mm) were used for rotatory chromatography and column chromatography, respectively. Melting points were determined with a hot-stage apparatus and are uncorrected. Optical rotations were measured at the sodium line at ambient temperature (26 °C). Mass spectra were carried out using electrospray ionization techniques (ESI-TOF) or electronic impact (EI); the latter was determined at 70 eV using an ion trap mass analyzer.

Nuclear magnetic resonance spectra were determined at 500 or 400 MHz for ^{1}H NMR and 125.7 or 100.6 MHz for ^{13}C NMR in the presence of tetramethylsilane (TMS) as the internal standard, at 25, 70, or 100 °C, as stated for each case. Because of rotamer equilibrium, the resolution of some NMR spectra at 26 °C was low (formation of broad bands); for those cases, the spectra at 70 or 100 °C are provided. *N*-alkyl amino acids in peptides make the interconversion of rotamers difficult, and thus, even at 70 °C, some minor rotamers can be observed. The NMR spectra at 70 and 100 °C of compound **25** are shown in the Supporting Information as examples; only at 100 °C, the signals coalesced, and only one rotamer was visible. The NMR signals were

DOI: 10.1021/acs.joc.9b00114
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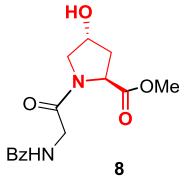
assigned with the help of COSY and HSQC experiments; in relevant cases, the 2D experiments are displayed in the Supporting Information.

^{1}H NMR references: $CDCl_3$ (δ_H 7.26), CD_3OD (δ_H 3.30), DMSO-d_6 (δ_H 2.50), C_6D_6 (δ_H 7.16); ^{13}C NMR references: $CDCl_3$ (δ_C 77.0), CD_3OD (δ_C 49.0); DMSO-d_6 (δ_C 39.5), C_6D_6 (δ_C 128.4). Abbreviations (in the NMR spectra): br b, broad band; br d, broad doublet; and so forth.

Preparation of Substrates for the Scission Process. Substrates (**6**) and (**7**) have been previously reported.[29b] The synthesis of compound (**8**) is commented below. Substrate (**32**) was prepared from intermediate (**40**), which was derived from commercial hydroxyproline methyl ester, as described in this section. Scission substrate (**37**) was synthesized from intermediates **41**−**45**, and the complete procedure is summarized in the scheme shown in the Supporting Information and described below.

*N-(N-Benzoyl-L-glycyl)-4R-hydroxy-L-proline Methyl Ester (**8**).*



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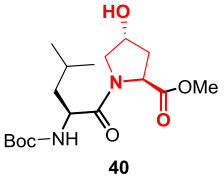
The commercial hippuric acid (**28**) [Bz-Gly-OH, 1.79 g, 10 mmol] was dissolved in CH_2Cl_2 (30 mL), and 4*R*-hydroxy-L-proline methyl ester hydrochloride (**22**) [H-Hyp-OMe·HCl, 2.36 g, 13 mmol], HBTU (4.17 g, 11 mmol), and DIPEA (5.1 mL, 3.87 g, 30 mmol) were added at 0 °C. The solution was stirred for 2 h; then, it was washed with saturated aqueous $NaHCO_3$ and 2% aqueous HCl and extracted with CH_2Cl_2. The organic layer was dried on sodium sulfate, filtered, and evaporated under vacuum. After purification by column chromatography (EtOAc/MeOH, 99:1), dipeptide **8** was isolated (2.70 g, 82%) as a syrup; $[\alpha]_D$ −53 (*c* 0.48, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3690, 3609, 3414, 1747, 1647, 1456, 1437 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, 26 °C): δ_H 2.08 (1H, m), 2.28 (1H, m), 3.64 (1H, br d, *J* = 10.7 Hz), 3.72 (3H, s), 3.80 (1H, m), 4.17 (1H, d, *J* = 16.8 Hz), 4.31 (1H, d, *J* = 17.0 Hz), 4.53 (1H, m), 4.55 (1H, ddd, *J* = 7.8, 7.9, 8.2 Hz), 7.47 (2H, dd, *J* = 7.6, 7.9 Hz), 7.55 (1H, dd, *J* = 6.9, 7.6 Hz), 7.86 (2H, d, *J* = 8.3 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 38.5 (CH_2), 43.2 (CH_2), 53.0 (CH_3), 55.5 (CH_2), 59.6 (CH), 71.2 (CH), 128.6 (2 × CH), 129.7 (2 × CH), 133.0 (CH), 135.3 (C), 170.1 (C), 170.4 (C), 174.4 (C); HRMS (EI): calcd for $C_{15}H_{18}N_2O_5Na$ (M^+ + Na), 329.1113; found, 329.1110. Anal. Calcd for $C_{15}H_{18}N_2O_5$: C, 58.82; H, 5.92; N, 9.15. Found: C, 58.98; H, 6.12; N, 8.96.

*N-[N-(tert-Butyloxycarbonyl)-L-leucyl]-4R-hydroxy-L-proline Methyl Ester (**40**), Precursor of Substrate (**32**).*

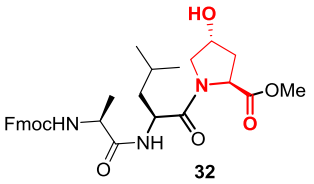


40

Commercial Boc-Leu-OH (2.3 g, 10 mmol) was dissolved in CH_2Cl_2 (30 mL), and 4*R*-hydroxy-L-proline methyl ester hydrochloride [H-Hyp-OMe·HCl, 2.36 g, 13 mmol], HBTU (4.17 g, 11 mmol), and DIPEA (3.4 mL, 2.58 g, 20 mmol) were added at 0 °C. The solution was stirred for 2 h; then, it was washed with saturated aqueous $NaHCO_3$ and 2% aqueous HCl. and extracted with CH_2Cl_2. The organic layer was dried on sodium sulfate, filtered, and evaporated under vacuum. After purification by column chromatography (hexanes/EtOAc, 4:6), dipeptide **40** was isolated (2.79 g, 78%) as a syrup.

$[\alpha]_D$ −76 (*c* 0.52, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3436, 1746, 1697, 1651, 1505, 1438 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 0.95 (3H, d, *J* = 6.9 Hz), 0.97 (3H, d, *J* = 6.3 Hz), 1.40 (9H, s), 1.38−1.60 (2H, m), 1.74 (1H, m), 2.00 (1H, m), 2.34 (2H, m), 3.67 (1H, dd, *J* = 3.0, 10.8 Hz), 3.72 (3H, s), 4.03 (1H, d, *J* = 11 Hz), 4.39 (1H, m), 4.53 (1H, m), 4.67 (1H, dd, *J* = 8.5, 8.6 Hz), 5.13 (1H, br d, *J* = 8.6 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 22.1 (CH_3), 23.0 (CH_3), 24.5 (CH), 28.3 (3 × CH_3), 37.6 (CH_2), 41.4 (CH_2), 50.3 (CH), 52.2 (CH_3), 55.1 (CH_2), 57.4 (CH), 70.3 (CH), 80.1 (C), 156.1 (C), 172.1 (C), 172.6 (C). HRMS (EI) *m*/*z*: calcd for $C_{17}H_{30}N_2O_6$ $[M]^+$, 358.2104; found, 358.2118. Anal. Calcd for $C_{17}H_{30}N_2O_6$: C, 56.97; H, 8.44; N, 7.82. Found: C, 56.78; H, 8.50; N, 7.85.

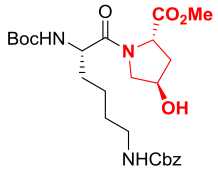
*N-[N-(Fluorenylmethyloxycarbonyl)-L-alanyl-L-leucyl]-4R-hydroxy-L-proline Methyl Ester (**32**).*



32

Dipeptide Boc-Leu-Hyp-OMe (**40**) (3.22 g, 9 mmol) was dissolved in CH_2Cl_2 (10 mL) at 0 °C and treated with trifluoroacetic acid (10 mL). The reaction mixture was allowed to reach 26 °C and stirred for 2 h; then, the volatiles were removed under vacuum. The crude dipeptide H-Leu-Hyp-OMe was not purified but dissolved in CH_2Cl_2 (40 mL). The solution was cooled to 0 °C and treated with amino acid Fmoc-Ala-OH (3.26 g, 10.5 mmol), DIPEA (3.1 mL, 18 mmol), EDC·HCl (2.07 g, 10.8 mmol), and HOBt hydrate (1.5 g, 11 mmol), and stirred for 1 h. The reaction mixture was allowed to reach 26 °C and stirred for another 16 h; then, it was poured into saturated aqueous $NaHCO_3$ and afterward, the organic layer was washed with 10% aqueous HCl. After usual drying and solvent removal, the residue was purified by column chromatography (hexanes/EtOAc, 1:9), yielding tripeptide Fmoc-Ala-Leu-Hyp-OMe (**32**) (3.37 g, 68%), as an amorphous solid; $[\alpha]_D$ −80 (*c* 0.29, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3429, 1745, 1718, 1651, 1505, 1451 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, 26 °C): δ_H 0.94 (6H, d, *J* = 6.3 Hz), 1.31 (3H, d, *J* = 7.3 Hz), 1.51−1.58 (2H, m), 1.73 (1H, m), 2.00 (1H, m), 2.23 (1H, m), 3.68 (3H, s), 3.72 (1H, dd, *J* = 4.1, 10.8 Hz), 3.80 (1H, br dd, *J* = 10.8, 12.6 Hz), 4.16 (1H, m), 4.21 (1H, m), 4.30−4.38 (2H, m), 4.48 (1H, m), 4.50 (1H, dd, *J* = 8.2, 8.9 Hz), 4.68 (1H, dd, *J* = 6.3, 8.2 Hz), 7.30 (2H, dd, *J* = 7.3, 7.3 Hz), 7.38 (2H, dd, *J* = 7.3, 7.6 Hz), 7.66 (2H, dd, *J* = 8.2, 9.5 Hz), 7.78 (2H, d, *J* = 7.6 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 18.2 (CH_3), 22.2 (CH_3), 23.5 (CH_3), 25.7 (CH), 38.3 (CH_2), 41.6 (CH_2), 48.5 (CH), 49.5 (CH), 50.7 (CH), 52.7 (CH_3), 56.3 (CH_2), 59.4 (CH), 68.1 (CH_2), 71.0 (CH), 120.9 (2 × CH), 126.2 (2 × CH), 128.2 (2 × CH), 128.8 (2 × CH), 142.6 (2 × C), 145.2 (C), 145.4 (C), 158.2 (C), 173.3 (C), 174.0 (C), 175.4 (C); HRMS (EI) *m*/*z*: calcd for $C_{30}H_{37}N_3O_7$ $[M]^+$, 551.2632; found, 551.2618. Anal. Calcd for $C_{30}H_{37}N_3O_7$: C, 65.32; H, 6.76; N, 7.62. Found: C, 65.10; H, 7.03; N, 7.56.

*N-(N^6-((Benzyloxy)carbonyl)-N^2-(tert-butoxycarbonyl)-L-lysyl)-4R-hydroxy-L-proline Methyl Ester (**42**), Precursor of the Scission Substrate (**37**).* A solution of Boc-Lys(Cbz)-OH (**41**) (1.14 g, 3 mmol)



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DOI: 10.1021/acs.joc.9b00114
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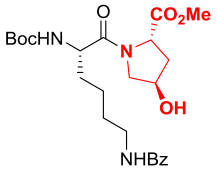
in CH_2Cl_2 (20 mL) at 0 °C was treated with 4*R*-hydroxy-L-proline methyl ester hydrochloride [H-Hyp-OMe·HCl, 654 mg, 3.6 mmol], HBTU (1.36 g, 3.6 mmol), and DIPEA (1.2 mL, 890 mg, 7.0 mmol). The solution was stirred for 2 h and then was poured into $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with 2% HCl, dried on sodium sulfate, filtered, and evaporated under vacuum. After purification by column chromatography (hexanes/EtOAc, 1:4), dipeptide **42** was isolated (1.03 g, 68%) as foam; $[\alpha]_D$ −31 (*c* 0.23, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3442, 1741, 1706, 1652, 1508, 1438 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 1.33−1.63 (5H, m), 1.38 (9H, s), 1.73 (1H, m), 1.94 (1H, m), 2.28 (1H, br dd, *J* = 8.2, 13.2 Hz), 3.17 (2H, dd, *J* = 5.7, 6.6 Hz), 3.61 (1H, m), 3.64 (3H, s), 3.83 (1H, br d, *J* = 10.4 Hz), 4.37 (1H, m), 4.46 (1H, m), 4.62 (1H, dd, *J* = 8.2, 8.6 Hz), 5.06 (2H, s), 5.33 (1H, br b), 5.44 (1H, br b), 7.27−7.34 (5H, m); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 21.5 (CH_2), 28.3 (3 × CH_3), 29.1 (CH_2), 31.7 (CH_2), 37.3 (CH_2), 40.4 (CH_2), 51.6 (CH), 52.3 (CH_3), 55.1 (CH_2), 57.5 (CH), 66.5 (CH_2), 70.1 (CH), 79.9 (C), 128.0 (2 × CH), 128.4 (3 × CH), 136.6 (C), 155.8 (C), 156.5 (C), 171.4 (C), 172.6 (C); HRMS (ESI-TOF): calcd for $C_{25}H_{37}N_3O_8Na$ (M^+ + Na), 530.2478; found, 530.2477. Anal. Calcd for $C_{25}H_{37}N_3O_8$: C, 59.16; H, 7.35; N, 8.28. Found: C, 59.20; H, 7.45; N, 8.20.

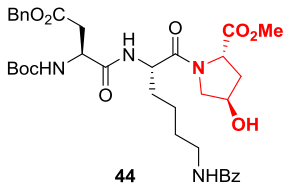
*N-(N^6-(Benzoyl)-N^2-(tert-butoxycarbonyl)-L-lysyl)-4R-hydroxy-L-proline Methyl Ester (**43**), Precursor of the Scission Substrate (**37**).*



43

Dipeptide Boc-Lys(Cbz)-Hyp-OMe (**42**) (1.01 g, 2 mmol) was dissolved in MeOH (20 mL) and treated with Pd (10% on carbon, 250 mg). The reaction mixture was stirred at room temperature and under hydrogen atmosphere (1 atm) for 16 h. Then, it was filtered through Celite, and the volatiles were removed under vacuum. Crude dipeptide Boc-Lys-Hyp-OMe was not purified but dissolved in CH_2Cl_2 (20 mL). The solution was cooled to 0 °C and treated with benzoic acid (293 mg, 2.4 mmol), HBTU (910 mg, 2.4 mmol), and DIPEA (820 μL, 610 mg, 4.8 mmol). The reaction mixture was stirred for 2 h; then, it was poured into $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with 2% aqueous HCl, dried on sodium sulfate, filtered, and evaporated under vacuum. After purification by column chromatography (EtOAc), dipeptide **43** was isolated (687 mg, 72%) as a syrup; $[\alpha]_D$ −32 (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3433, 3025, 1743, 1699, 1652, 1506 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 1.38 (9H, s), 1.40−1.53 (2H, m), 1.58−1.71 (3H, m), 1.79 (1H, m), 1.88 (1H, ddd, *J* = 4.4, 8.8, 13.2 Hz), 2.30 (1H, dd, *J* = 8.2, 13.2 Hz), 3.08 (1H, br b), 3.45 (2H, ddd, *J* = 5.4, 5.5, 5.7 Hz), 3.62 (1H, m), 3.64 (3H, s), 3.86 (1H, d, *J* = 10.7 Hz), 4.38 (1H, m), 4.48 (1H, m), 4.63 (1H, dd, *J* = 8.2, 8.5 Hz), 5.41 (1H, br b), 6.77 (1H, br b), 7.38 (2H, dd, *J* = 7.3, 7.6 Hz), 7.45 (1H, dd, *J* = 7.3, 7.6 Hz), 7.79 (2H, d, *J* = 8.2 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 21.8 (CH_2), 28.3 (3 × CH_3), 28.7 (CH_2), 31.8 (CH_2), 37.3 (CH_2), 39.6 (CH_2), 51.5 (CH), 52.3 (CH_3), 55.2 (CH_2), 57.6 (CH), 70.1 (CH), 80.0 (C), 127.1 (2 × CH), 128.3 (2 × CH), 131.3 (CH), 134.6 (C), 155.8 (C), 167.9 (C), 171.4 (C), 172.7 (C); HRMS (ESI-TOF): calcd for $C_{24}H_{35}N_3O_7Na$ (M^+ + Na), 500.2373; found, 500.2367. Anal. Calcd for $C_{24}H_{35}N_3O_7$: C, 60.36; H, 7.39; N, 8.80. Found: C, 60.05; H, 7.71; N, 8.50.

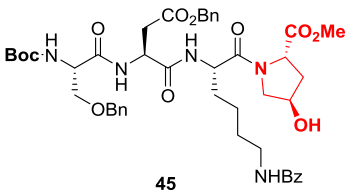
*N-(N^6-(Benzoyl)-N^2-(N-tert-butoxycarbonyl-O-benzyl-L-aspartyl)-L-lysyl)-4R-hydroxy-L-proline Methyl Ester (**44**), Precursor of the Scission Substrate (**37**).*



44

Dipeptide Boc-Lys(Bz)-Hyp-OMe (**43**) (954 mg, 2 mmol) was dissolved in CH_2Cl_2 (10 mL) at 0 °C and treated with trifluoroacetic acid (10 mL). The reaction mixture was allowed to reach 26 °C and stirred for 2 h; then, the volatiles were removed under vacuum. Crude dipeptide H-Lys(Bz)-Hyp-OMe was dissolved in CH_2Cl_2 (20 mL), and Boc-Asp(Bzl)-OH (775 mg, 2.4 mmol), HBTU (910 mg, 2.4 mmol), and DIPEA (820 μL, 610 mg, 4.8 mmol) were added at 0 °C. The solution was stirred for 2 h; then, it was poured into $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with 2% HCl, dried on sodium sulfate, filtered, and evaporated under vacuum. After purification by column chromatography (EtOAc), tripeptide **44** was isolated (0.98 g, 72%) as foam; $[\alpha]_D$ −29 (*c* 0.17, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3420, 1734, 1717, 1652, 1648, 1522, 1489 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 1.39−1.49 (2H, m), 1.42 (9H, s), 1.58−1.72 (3H, m), 1.83−1.94 (2H, m), 2.24 (1H, m), 2.31 (1H, m), 2.73 (1H, dd, *J* = 4.7, 17.0 Hz), 2.97 (1H, dd, *J* = 3.9, 17.0 Hz), 3.44 (2H, m), 3.64 (1H, m), 3.66 (3H, s), 3.82 (1H, d, *J* = 10.7 Hz), 4.47 (2H, m), 4.63 (1H, dd, *J* = 8.2, 8.2 Hz), 4.67 (1H, ddd, *J* = 6.5, 6.9, 6.9 Hz), 5.03 (1H, d, *J* = 12.6 Hz), 5.07 (1H, d, *J* = 12.5 Hz), 5.57 (1H, br d, *J* = 8.2 Hz), 6.76 (1H, br b), 7.23 (1H, br d, *J* = 6.9 Hz), 7.28−7.37 (7H, m), 7.45 (1H, dd, *J* = 6.7, 8.2 Hz), 7.81 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, 26 °C): δ_C 21.6 (CH_2), 28.2 (3 × CH_3), 28.5 (CH_2), 31.3 (CH_2), 36.0 (CH_2), 37.3 (CH_2), 39.5 (CH_2), 50.7 (CH), 50.8 (CH), 52.3 (CH_3), 55.3 (CH_2), 57.6 (CH), 66.8 (CH_2), 70.1 (CH), 80.7 (C), 127.1 (2 × CH), 128.1 (2 × CH), 128.3 (3 × CH), 128.5 (2 × CH), 131.2 (CH), 134.7 (C), 135.2 (C), 155.4 (C), 167.9 (C), 170.3 (C), 170.6 (C), 171.6 (C), 172.6 (C); HRMS (ESI-TOF): calcd for $C_{35}H_{46}N_4O_{10}Na$ (M^+ + Na), 705.3112; found, 705.3116. Anal. Calcd for $C_{35}H_{46}N_4O_{10}$: C, 61.57; H, 6.79; N, 8.21. Found; C, 61.44; H, 6.91; N, 8.12.

*N-[N^2-(N-tert-Butoxycarbonyl-O-benzyl-L-Seryl-O-benzyl-L-aspartyl)-N^6-(benzoyl)-L-lysyl]-4R-hydroxy-L-proline Methyl Ester (**45**), Precursor of the Scission Substrate (**37**).*



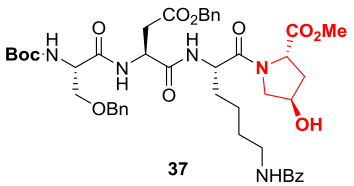
45

Tripeptide Boc-Asp(Bzl)-Lys(Bz)-Hyp-OMe (**44**) (1.36 g, 2 mmol) was dissolved in CH_2Cl_2 (10 mL) at 0 °C and treated with trifluoroacetic acid (10 mL). The reaction mixture was allowed to reach 26 °C and stirred for 2 h, then the volatiles were removed under vacuum. Crude tripeptide H-Asp(Bzl)-Lys(Bz)-Hyp-OMe was dissolved in CH_2Cl_2 (20 mL), and the solution was cooled to 0 °C and treated with Boc-Ser(Bzl)-OH (708 mg, 2.4 mmol), HBTU (910 mg, 2.4 mmol), and DIPEA (820 μL, 610 mg, 4.8 mmol). The reaction mixture was stirred for 2 h; then, it was poured into $NaHCO_3$ and extracted with CH_2Cl_2.

DOI: 10.1021/acs.joc.9b00114
J. Org. Chem. 2019, 84, 8392−8410

The organic layer was washed with 2% HCl, dried on sodium sulfate, filtered, and evaporated under vacuum. After purification by column chromatography (EtOAc), tetrapeptide **45** was isolated (1.22 g, 71%) as a syrup; $[\alpha]_D$ −25 (*c* 0.29, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3420, 3068, 1733, 1718, 1654, 1508 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 1.34−1.46 (2H, m), 1.42 (9H, s), 1.50−1.62 (3H, m), 1.80 (1H, m), 1.87 (1H, ddd, *J* = 4.4, 9.5, 13.6 Hz), 2.30 (1H, dd, *J* = 7.9, 13.2 Hz), 2.67 (1H, dd, *J* = 5.0, 17.0 Hz), 3.01 (1H, dd, *J* = 4.4, 17.0 Hz), 3.36 (1H, m), 3.44 (1H, m), 3.56−3.65 (2H, m), 3.61 (3H, s), 3.70−3.80 (3H, m), 4.20 (1H, m), 4.46 (1H, m), 4.50 (1H, d, *J* = 12.0 Hz), 4.53 (1H, d, *J* = 12.0 Hz), 4.56 (1H, m), 4.62 (1H, dd, *J* = 8.5, 8.8 Hz), 4.74 (1H, m), 5.01 (1H, d, *J* = 12.0 Hz), 5.05 (1H, d, *J* = 12.3 Hz), 5.37 (1H, br b), 6.92 (1H, br b), 7.25−7.45 (14H, m), 7.63 (1H, br d, *J* = 8.2 Hz), 7.81 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 21.8 (CH_2), 28.2 (3 × CH_3), 28.4 (CH_2), 30.7 (CH_2), 35.1 (CH_2), 37.3 (CH_2), 39.3 (CH_2), 49.8 (CH), 50.9 (CH), 52.2 (CH_3), 54.7 (CH), 55.1 (CH_2), 57.7 (CH), 66.8 (CH_2), 69.3 (CH_2), 70.2 (CH), 73.3 (CH_2), 80.7 (C), 127.1 (2 × CH), 127.7 (2 × CH), 127.9 (CH), 128.2 (2 × CH), 128.3 (3 × CH), 128.4 (2 × CH), 128.5 (2 × CH), 131.1 (CH), 134.8 (C), 135.2 (C), 137.2 (C), 155.8 (C), 167.6 (C), 169.8 (C), 169.9 (C), 170.9 (C), 171.4 (C), 172.7 (C); HRMS (ESI-TOF): calcd for $C_{45}H_{57}N_5O_{12}Na$ (M^+ + Na), 882.3901; found, 882.3909. Anal. Calcd for $C_{45}H_{57}N_5O_{12}$: C, 62.85; H, 6.68; N, 8.14. Found: C, 62.94; H, 6.76; N, 8.28.

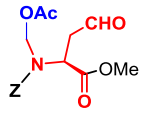
*N-[N²-(N-Acetyl-O-benzyl-L-seryl-O-benzyl-L-aspartyl)-N⁶-(benzoyl)-L-lysyl]-4R-hydroxy-L-proline Methyl Ester (**37**).*



37

Tetrapeptide Boc-Ser(Bzl)-Asp(Bzl)-Lys(Bz)-Hyp-OMe (**45**) (1.72 g, 2 mmol) was dissolved in CH_2Cl_2 (10 mL) at 0 °C and treated with trifluoroacetic acid (10 mL). The reaction mixture was allowed to reach 26 °C and stirred for 2 h; then, the volatiles were removed under vacuum. Crude tripeptide H-Ser(Bzl)-Asp(Bzl)-Lys(Bz)-Hyp-OMe was dissolved in tetrahydrofuran (THF) (15 mL), and DIPEA (685 μL, 508 mg, 4.0 mmol), Ac_2O (378 μL, 408 mg, 4.0 mmol), and H_2O (15 mL) were added at 0 °C. The solution was stirred for 16 h, while allowing it to reach 26 °C; then, it was poured into 5% aqueous HCl and extracted with EtOAc. The organic layer was dried on sodium sulfate, filtered, and evaporated under vacuum. After purification by column chromatography (CH_2Cl_2/MeOH, 98:2), the tetrapeptide (**37**) was isolated (1.03 g, 64%) as a crystalline solid: mp 123−124 °C (CH_2Cl_2); $[\alpha]_D$ −28 (*c* 0.24, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3420, 1741, 1734, 1718, 1653, 1648, 1455 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 26 °C): δ_H 1.42−1.47 (2H, m), 1.56−1.66 (3H, m), 1.79 (1H, m), 1.96 (1H, m), 1.97 (3H, s), 2.21 (1H, m), 2.81 (1H, dd, *J* = 7.3, 16.7 Hz), 2.90 (1H, dd, *J* = 5.5, 16.6 Hz), 3.32−3.39 (2H, m), 3.63 (3H, s), 3.65−3.77 (4H, m), 4.44 (1H, m), 4.49 (1H, dd, *J* = 8.4, 8.4 Hz), 4.58 (1H, d, *J* = 12 Hz), 4.59 (1H, dd, *J* = 5.8, 7.6 Hz), 4.61 (1H, d, *J* = 12 Hz), 4.80 (1H, dd, *J* = 5.7, 7.3 Hz), 5.06 (1H, d, *J* = 12.5 Hz), 5.09 (1H, d, *J* = 12.5 Hz), 7.23 (1H, m), 7.27−7.33 (9H, m), 7.41 (2H, dd, *J* = 7.3, 7.7 Hz), 7.49 (1H, dd, *J* = 7.4, 8.0 Hz), 7.80 (2H, d, *J* = 8.1 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 22.5 (CH_3), 23.5 (CH_2), 30.0 (CH_2), 32.2 (CH_2), 36.7 (CH_2), 38.3 (CH_2), 40.7 (CH_2), 51.1 (CH), 52.4 (CH), 52.7 (CH_3), 55.0 (CH), 56.3 (CH_2), 59.4 (CH), 67.7 (CH_2), 70.5 (CH_2), 71.0 (CH), 74.2 (CH_2), 128.3 (2 × CH), 128.8 (CH), 128.9 (2 × CH), 129.2 (3 × CH), 129.4 (2 × CH), 129.5 (2 × CH), 129.6 (2 × CH), 132.5 (CH), 135.9 (C), 137.3 (C), 139.2 (C), 170.2 (C), 172.0 (2 × C), 172.3 (C), 172.4 (C), 173.5 (C), 174.0 (C); HRMS (ESI-TOF): calcd for $C_{42}H_{51}N_5O_{11}Na$ (M^+ + Na), 824.3483; found, 824.3488. Anal. Calcd for $C_{42}H_{51}N_5O_{11}$: C, 62.91; H, 6.41; N, 8.73. Found: C, 62.88; H, 6.69; N, 8.95.

General Procedure for the Scission Process.



9 Z = Bz

10 Z = Phe-Cbz

11 Z = Gly-Bz

To a solution of the starting material (0.2 mmol) in dry dichloroethane (4 mL) was added iodine (51 mg, 0.2 mmol) and diacetoxyiodobenzene (DIB) (129 mg, 0.4 mmol). The reaction mixture was stirred at reflux (80−84 °C) until disappearance of the starting material (30−60 min) under irradiation with visible light (80 W tungsten lamp). Then, it was poured into 10% aqueous $Na_2S_2O_3$ and extracted with CH_2Cl_2. After drying over sodium sulfate, the organic layer was filtered and evaporated under vacuum. The residue was purified by chromatography on silica gel (hexanes/EtOAc) to afford *N*-acetoxymethyl 4-oxohomoalanine derivatives **9**−**11**.

*(2S)-N-(Acetoxymethyl)-N-benzoyl-4-oxo-L-homoalanine Methyl Ester (**9**).* It was obtained from *N*-benzoyl-L-hydroxyproline methyl ester (50 mg, 0.2 mmol) using the general scission protocol, but adding a smaller amount of iodine (25 mg, 0.1 mmol), and stirring for 30 min. After purification by rotatory chromatography (hexanes/EtOAc 80:20), homoalanine derivative **9** was obtained (49 mg, 80%) as a yellowish oil: $[\alpha]_D$ −78 (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3032, 1744, 1658, 1426, 1351, 1200 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 2.11 (3H, s), 3.23 (1H, dd, *J* = 7.6, 18.8 Hz), 3.50 (1H, br d, *J* = 16.7 Hz), 3.77 (3H, s), 4.94 (1H, dd, *J* = 5.3, 7.7 Hz), 5.41 (2H, br s), 7.36−7.52 (5H, m), 9.81 (1H, s); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 20.9 (CH_3), 44.1 (CH_2), 52.9 (CH_3), 54.9 (CH), 74.3 (CH_2), 127.4 (2 × CH), 128.5 (2 × CH), 130.9 (CH), 134.1 (C), 170.0 (C), 170.5 (C), 172.5 (C), 198.8 (CH). HRMS (ESI-TOF): calcd for $C_{15}H_{17}NO_6Na$ (M^+ + Na), 330.0954; found, 330.0954. Anal. Calcd for $C_{15}H_{17}NO_6$: C, 58.63; H, 5.58; N, 4.56. Found: C, 58.32; H, 5.94; N, 4.43.

*N-Acetoxymethyl-N-(N-benzyloxycarbonyl-L-phenylalanyl)-4-oxo-L-homoalanine Methyl Ester (**10**).* It was obtained from dipeptide Cbz-Phe-Hyp-OMe (**7**) (86.0 mg, 0.2 mmol) using the general scission protocol and stirring for 60 min. After purification by rotatory chromatography (hexanes/EtOAc 70:30), homoalanine derivative **10** was obtained (81 mg, 82%). Product **10** was previously described.[29b]

*(2S)-N-(Acetoxymethyl)-N-[N-(benzoyl)glycyl]-4-oxo-L-homoalanine Methyl Ester (**11**).* It was obtained from *N*-(benzoyl)glycyl-L-hydroxyproline methyl ester (**8**) (61 mg, 0.2 mmol) using the general scission protocol and stirring for 60 min. After purification by rotatory chromatography (hexanes/EtOAc 50:50), homoalanine derivative **11** was obtained (52 mg, 71%) as a syrup: $[\alpha]_D$ −79 (*c* 0.36, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3424, 1747, 1727, 1655, 1517, 1242 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 2.10 (3H, s), 3.19 (1H, dd, *J* = 8.2, 19.2 Hz), 3.45 (1H, dd, *J* = 4.7, 19.2 Hz), 3.70 (3H, s), 4.50 (2H, d, *J* = 4.1 Hz), 4.86 (1H, dd, *J* = 4.8, 8.2 Hz), 5.39 (1H, d, *J* = 12.3 Hz), 5.63 (1H, d, *J* = 12.0 Hz), 7.03 (1H, br b), 7.43 (2H, dd, *J* = 7.3, 7.8 Hz), 7.50 (1H, dd, *J* = 7.3, 7.3 Hz), 7.81 (2H, d, *J* = 7.6 Hz), 9.75 (1H, s); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 20.6 (CH_3), 41.7 (CH_2), 44.1 (CH_2), 52.9 (CH_3), 55.8 (CH), 71.5 (CH_2), 127.0 (2 × CH), 128.6 (2 × CH), 131.8 (CH), 133.6 (C), 167.2 (C), 169.6 (C), 170.2 (C),

DOI: 10.1021/acs.joc.9b00114
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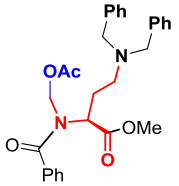
170.5 (C), 198.7 (CH). HRMS (ESI-TOF): calcd for $C_{17}H_{20}N_2O_7Na$ (M^+ + Na), 387.1168; found, 387.1169.

General Procedure for the Reductive Amination Process. To a solution of the aldehyde (0.15 mmol) in dry dichloroethane (3 mL) was added the amine (0.17 mmol) and Et_3N (28 μL, 0.2 mmol), and the mixture was stirred at 26 °C for 30 min before adding $NaBH(OAc)_3$ (95 mg, 0.45 mmol) and stirring for another 18 h. Then, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. After usual drying and evaporation of the organic layer, the residue was purified by chromatography on silica gel (hexanes/EtOAc) to give 4-amino-L-homoalanine derivatives.

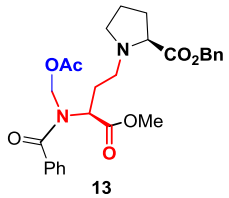
*(2S)-N-(Acetoxymethyl)-N-(benzoyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**12**).*



12

It was obtained from aldehyde **9** (46 mg, 0.15 mmol) on treatment with dibenzylamine (33 μL, 0.17 mmol) according to the general reductive amination protocol. After purification by rotatory chromatography on silica gel (hexanes/EtOAc, 85:15), compound **12** was obtained (76.5 mg, 92%) as a white crystalline solid: mp 76−78 °C (CH_2Cl_2); $[\alpha]_D$ −74 (*c* 0.37, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3066, 1741, 1659, 1494, 1347 cm^{-1}; ^1H NMR (400 MHz, CD_3OD, 26 °C): δ_H 1.95 (3H, s), 2.11 (1H, m), 2.44 (1H, m), 2.53 (1H, m), 2.65 (1H, m), 3.47 (2H, d, *J* = 13.3 Hz), 3.65 (3H, s), 3.66 (2H, d, *J* = 13.3 Hz), 4.75 (1H, m), 4.94 (1H, br d, *J* = 11.4 Hz), 5.12 (1H, br d, *J* = 11.0 Hz), 7.20 (4H, dd, *J* = 7.2, 7.2 Hz), 7.28 (4H, dd, *J* = 7.3, 7.4 Hz), 7.34−7.43 (6H, m), 7.49 (1H, dd, *J* = 7.2, 7.2 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 20.7 (CH_3), 27.8 (CH_2), 50.7 (CH_2), 52.8 (CH_3), 58.6 (CH), 59.6 (2 × CH_2), 74.8 (CH_2), 128.2 (2 × CH), 128.3 (2 × CH), 129.4 (4 × CH), 129.5 (2 × CH), 130.3 (4 × CH), 131.8 (CH), 135.9 (C), 140.7 (2 × C), 171.7 (C), 172.9 (C), 175.1 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{29}H_{32}N_2O_5Na$ [M + Na]$^+$, 511.2209; found, 511.2203. Anal. Calcd for $C_{29}H_{32}N_2O_5$: C, 71.29; H, 6.60; N, 5.73. Found: C, 71.52; H, 6.61; N, 5.87.

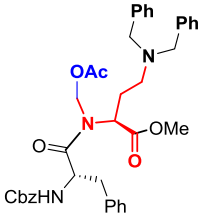
*N-(Benzoyl)-N-(acetoxymethyl)-4-[O-benzyl-L-prolin-1-yl]-L-homoalanine methyl ester (**13**).*



13

It was obtained from aldehyde **9** (46 mg, 0.15 mmol) on treatment with L-proline benzyl ester hydrochloride (41 mg, 0.17 mmol) according to the general reductive amination protocol. After purification by rotatory chromatography on silica gel (hexanes/EtOAc, 85:15), compound **13** was obtained (58 mg, 78%) as a syrup; $[\alpha]_D$ −78 (*c* 0.63, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3090, 3067, 1741, 1654, 1238, 1015 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C): δ_H 1.79−1.87 (2H, m), 1.91 (1H, m), 2.02 (3H, s), 2.12 (1H, m), 2.18 (1H, m), 2.32 (1H, m), 2.43 (1H, ddd, *J* = 7.9, 8.1, 8.5 Hz), 2.56 (1H, ddd, *J* = 4.8, 7.6, 12.0 Hz), 2.90 (1H, ddd, *J* = 7.9, 8.2, 12.0 Hz), 3.13 (1H, m), 3.29 (1H, m), 3.73 (3H, s), 4.66 (1H, dd, *J* = 5.0, 9.5 Hz), 5.11 (1H, d, *J* = 12.3 Hz), 5.14 (1H, d, *J* = 12.3 Hz), 5.30 (1H, br d, *J* = 10.8 Hz), 5.41 (1H, d, *J* = 11.7 Hz), 7.27−7.34 (4H, m), 7.41−7.51 (6H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 70 °C): δ_C 20.7 (CH_3), 24.1 (CH_2), 29.3 (CH_2), 30.2 (CH_2), 52.0 (CH_2), 52.8 (CH_3), 53.9 (CH_2), 59.5 (CH), 67.4 (CH_2), 74.5 (CH_2), 128.2 (2 × CH), 129.15 (2 × CH), 129.24 (2 × CH), 129.5 (3 × CH), 131.6 (CH), 136.4 (C), 137.6 (C), 171.9 (C), 172.9 (C), 175.2 (C), 175.3 (C). A broad CH signal about δ_C 50 was overlapped with the solvent. HRMS (ESI-TOF) *m*/*z*: calcd for $C_{27}H_{33}N_2O_7$ [M + H]$^+$, 497.2288; found, 497.2286. Anal. Calcd for $C_{27}H_{32}N_2O_7$: C, 65.31; H, 6.50; N, 5.64. Found: C, 65.39; H, 6.42; N, 5.87.

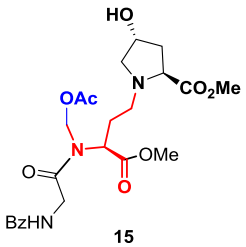
*(2S)-N-(Acetoxymethyl)-N-[N-(benzyloxycarbonyl)-L-phenylalanyl]-4-dibenzylamino-L-homoalanine Methyl Ester (**14**).*



14

It was obtained from aldehyde **10** (73 mg, 0.15 mmol) on treatment with dibenzylamine (33 μL, 0.17 mmol) according to the general reductive amination protocol. After purification by rotatory chromatography on silica gel (hexanes/EtOAc, 95:5), compound **14** was obtained (84 mg, 84%) as a syrup; $[\alpha]_D$ −26 (*c* 0.45, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3430, 1742, 1719, 1671, 1508, 1497 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C): δ_H 1.80 (1H, m), 1.91 (3H, s), 2.27 (1H, m), 2.35−2.45 (2H, m), 2.80 (1H, dd, *J* = 8.0, 13.2 Hz), 2.90 (1H, dd, *J* = 7.0, 13.6 Hz), 3.46 (2H, d, *J* = 13.2 Hz), 3.49 (3H, s), 3.51 (2H, d, *J* = 13.6 Hz), 4.55 (1H, br b), 4.61 (1H, dd, *J* = 5.7, 7.6 Hz), 4.87 (1H, dd, *J* = 6.7, 7.9 Hz), 4.98 (2H, br s), 5.04 (1H, d, *J* = 12.3 Hz), 5.25 (1H, d, *J* = 12.3 Hz), 7.15−7.35 (20H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 20.9 (CH_3), 28.3 (CH_2), 39.9 (CH_2), 51.3 (CH_2), 52.8 (CH_3), 54.1 (CH), 59.4 (CH), 59.6 (2 × CH_2), 67.7 (CH_2), 72.7 (CH_2), 128.1 (CH), 128.3 (2 × CH), 128.8 (2 × CH), 129.1 (CH), 129.5 (4 × CH), 129.6 (2 × CH), 129.7 (2 × CH), 130.4 (4 × CH), 130.7 (2 × CH), 138.1 (C), 138.3 (C), 140.8 (2 × C), 158.0 (C), 172.1 (C), 172.8 (C), 175.3 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{39}H_{43}N_3O_7Na$ [M + Na]$^+$, 688.2999; found, 688.2993. Anal. Calcd for $C_{39}H_{43}N_3O_7$: C, 70.36; H, 6.51; N, 6.31. Found: C, 70.27; H, 6.61; N, 6.11.

*N-(N-Benzoyl-L-glycyl)-N-(acetoxymethyl)-4-[4R-hydroxy-O-Methyl-L-prolin-1-yl]-L-homoalanine Methyl Ester (**15**).*



15

It was obtained from aldehyde **11** (46 mg, 0.15 mmol) on treatment with 4*R*-hydroxy-L-proline methyl ester hydrochloride (31 mg, 0.17 mmol) according to the general reductive amination protocol. After purification by rotatory chromatography on silica gel (hexanes/EtOAc, 30:70), compound **15** was obtained (63 mg, 85%) as an oil; $[\alpha]_D$ −80 (*c* 0.24, MeOH); IR ($CHCl_3$) ν_{max}: 3623, 3420, 1744, 1654, 1520 cm^{-1}; ^1H NMR

DOI: 10.1021/acs.joc.9b00114
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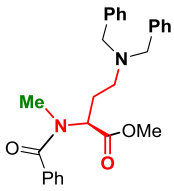
(500 MHz, $CDCl_3$, 26 °C) 3:1 rotamer mixture; minor rotamer/major rotamer: δ_H ***2.01***/2.08 (3H, ***s***/s), 2.10−2.37 (4H, m), ***2.49***/2.55 (1H, ***m***/m), 2.59−2.70 (1H, m), 2.80−2.95 (1H, m), ***3.42***/3.46 (1H, ***m***/m), ***3.61***/3.68 (3H, ***s***/s), 3.69 (1H, m), 3.69/***3.74*** (3H, s/***s***), ***4.20***/4.49 (1H, [***dd, J = 3.5, 17.7 Hz***/dd, *J* = 4.4, 17.5 Hz]), 4.47/***4.52*** (1H, m/***m***), 4.60/***4.79*** (1H, [dd, *J* = 4.8, 10.1 Hz/***dd, J = 3.8, 14.2 Hz***]), 4.56/***4.91*** (1H, [dd, *J* = 4.8, 17.7 Hz/***dd, J = 4.8, 17.5 Hz***]), ***5.29***/5.44 (1H, [d, *J* = 12.3 Hz/***d, J = 12.3 Hz***]), 5.62/***5.68*** (1H, [d, *J* = 10.7 Hz/***d, J = 12.6 Hz***]), ***7.17***/7.23 (1H, [***br b***/br b]), 7.42 (2H, dd, *J* = 7.0/7.3 Hz), 7.49 (1H, dd, *J* = 7.2, 7.5 Hz), 7.81 (2H, d, *J* = 7.8 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C) 3:1 rotamer mixture; minor rotamer/major rotamer: δ_C 20.7 (CH_3), 27.2/***27.9*** (CH_2), 39.1/***39.3*** (CH_2), 41.8/***42.2*** (CH_2), ***47.7***/50.4 (CH_2), ***51.7***/52.0 (CH_3), 52.5/***52.8*** (CH_3), ***55.1***/58.7 (CH), 60.8 (CH_2), ***63.3***/64.1 (CH), 69.8/***69.9*** (CH), ***67.6***/71.6 (CH_2), 127.1 (2 × CH), 128.5 (2 × CH), 131.7 (CH), 133.7 (C), 167.4 (C), ***170.0***/170.4 (C), 170.7 (C), 170.9/***171.2*** (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{23}H_{32}N_3O_9$ $[M + H]^+$, 494.2139; found, 494.2133. Anal. Calcd for $C_{23}H_{31}N_3O_9$: C, 55.98; H, 6.33; N, 8.51. Found: C, 55.72; H, 6.28; N, 8.14.

General Procedure for the Reduction of the *N*-Acetoxymethyl Group. A solution of the substrate (0.1 mmol) in dry MeCN was cooled to 0 °C and treated with Et_3SiH (58 mg, 0.5 mmol) and TMS-OTf (37 μL, 0.2 mmol). The reaction mixture was stirred until complete conversion of the starting material (1−3 h). Then, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was dried, and the solvent was removed as usual. The residue was purified by rotatory chromatography on silica gel (hexanes/EtOAc) to give the *N*-methyl amino acid derivatives.

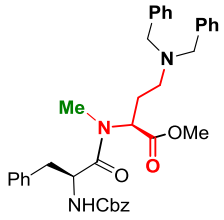
*(2S)-N-(Methyl)-N-(benzoyl)-4-dibenzylamino-L-homoalanine (**16**).*



16

It was obtained from the *N*-acetoxymethyl derivative (**12**) (49 mg, 0.1 mmol) according to the general reduction procedure. After 1 h, usual work-up and solvent evaporation afforded a residue that was purified by rotatory chromatography (hexanes/EtOAc, 90:10) yielding product **16** (34 mg, 79%) as a crystalline solid: mp 96−98 °C (AcOEt). $[\alpha]_D$ −16 (*c* 0.36, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3066, 1739, 1632, 1495 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C) 2:1 rotamer mixture at 26 °C, one rotamer at 70 °C: δ_H 1.95 (1H, m), 2.27 (1H, m), 2.52−2.57 (2H, m), 2.71 (3H, br s), 3.50−3.70 (4H, m), 3.64 (3H, s), 4.99 (1H, m), 7.10−7.49 (15H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 27.4/28.4 (CH_2), 36.0 (CH_3), 50.7/50.8 (CH_2), 52.8/52.9 (CH_3), 59.6/59.8 (2 × CH_2), 57.2/61.0 (CH), 128.1 (2 × CH), 128.3 (2 × CH), 129.4 (2 × CH), 129.5 (3 × CH), 129.7 (2 × CH), 130.1 (2 × CH), 130.3 (2 × CH), 131.3 (CH), 136.9 (C), 140.1/140.8 (2 × C), 172.3/173.0 (C), 174.8 (C). HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$: calcd for $C_{27}H_{30}N_2O_3Na$, 453.2154; found, 453.2152. Anal. Calcd for $C_{27}H_{30}N_2O_3$: C, 75.32; H, 7.02; N, 6.51. Found: C, 75.04; H, 7.17; N, 6.63.

*(2S)-N-(Methyl)-N-[N-(benzyloxycarbonyl)-L-phenylalanyl]-4-dibenzylamino-L-homoalanine Methyl Ester (**17**).* It was obtained from the *N*-acetoxymethyl derivative (**14**) (67 mg, 0.1 mmol) according to the general reduction procedure. After 3 h, usual work-up and solvent evaporation afforded a residue that was purified by rotatory

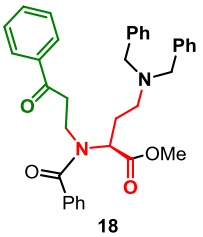


17

chromatography (hexanes/EtOAc, 80:20) yielding product (**17**) (49 mg, 81%) as a yellowish oil. $[\alpha]_D$ −10 (*c* 0.30, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3428, 1737, 1715, 1648, 1496, 1454 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C) 4:1 rotamer mixture at 70 °C. Minor rotamer/major rotamer: δ_H ***1.61***/1.73 (1H, ***m***/m), 2.11 (1H, m), 2.30−2.50 (2H, m), ***2.63***/2.71 (3H, s), 2.84 (1H, dd, *J* = 7.3, 13.6 Hz), 2.96 (1H, dd, *J* = 6.8, 13.4 Hz), 3.48 (2H, d, *J* = 13.3 Hz), 3.53 (2H, d, *J* = 13.3 Hz), 3.54 (3H, s), 4.74 (1H, m), 4.84 (1H, m), 4.95 (1H, d, *J* = 12.6 Hz), 4.99 (1H, d, *J* = 12.3 Hz), 7.10−7.40 (20H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 27.7 (CH_2), 33.7 (CH_3), 39.1 (CH_2), 51.4 (CH_2), 52.7 (CH_3), 54.0 (CH), 57.9 (CH), 59.6 (2 × CH_2), 67.6 (CH_2), 128.1 (CH), 128.2 (2 × CH), 128.8 (2 × CH), 129.0 (CH), 129.3 (4 × CH), 129.5 (2 × CH), 129.7 (2 × CH), 130.3 (4 × CH), 130.6 (2 × CH), 138.0 (C), 138.3 (C), 140.8 (2 × C), 158.1 (C), 172.8 (C), 174.3 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{37}H_{42}N_3O_5$ $[M + H]^+$, 608.3124; found, 608.3125. Anal. Calcd for $C_{37}H_{41}N_3O_5$: C, 73.12; H, 6.80; N, 6.91. Found: C, 73.03; H, 6.94; N, 6.83.

General Procedure for the Addition of *C*-Nucleophiles. A solution of the substrate (0.1 mmol) in dry MeCN was cooled to 0 °C and treated with the *C*-nucleophile (0.5 mmol) and TMS-OTf (37 μL, 0.2 mmol). The reaction mixture was stirred for 5 h, and then, it was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was dried, and the solvent was removed as usual. The residue was purified by rotatory chromatography on silica gel (hexanes/EtOAc) to give the *N*-alkyl amino acid derivatives.

*N-(Benzoyl)-N-(3-oxo-3-phenylpropyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**18**).*



18

It was obtained from the *N*-acetoxymethyl derivative (**12**) (49 mg, 0.1 mmol) using 1-phenyl-1-trimethylsiloxyethylene (96 mg, 102 μL, 0.5 mmol) according to the general addition procedure. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 90:10), yielding product (**18**) (52 mg, 95%) as a syrup. $[\alpha]_D$ −51 (*c* 0.85, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 1739, 1682, 1634, 1449 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 26 °C) 1:1 rotamer mixture at 26 °C: δ_H 1.90/2.10 (1H, m/m), 2.33/2.48 (1H, m/m), 2.50 (1H, m/m), 2.61/2.68 (1H, m/m), 3.30 (1H, m), 3.45 (1H, m), 3.55 (3H, s), 3.56−3.67 (4H, m), 4.10/4.30 (1H, m), 4.59 (2H, m), 7.07−7.65 (18H, m), 7.82/8.01 (2H, [d, *J* = 6.3 Hz/d, *J* = 6.0 Hz]). The signals at δ_H 4.10/4.30 are broad, flat bands which do not properly integrate for 1H, but heteronuclear single quantum coherence (HSQC) shows that they both correspond to the signals at δ_H 59.8/59.9; ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 28.0/29.1 (CH_2), 48.5 (CH_2), 50.9/51.2 (CH_2), 52.8/53.1 (CH_3), 59.5/59.7 (CH_2), 59.8/59.9 (CH), 61.5 (2 × CH_2), 127.6/128.0 (2 × CH), 128.3 (CH), 129.2/129.5 (4 × CH), 129.9 (6 × CH), 130.3/130.5 (2 × CH), 131.1/131.2

DOI: 10.1021/acs.joc.9b00114
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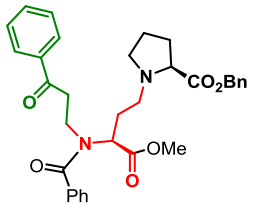
(CH), 134.6 (2 × CH), 137.3 (2 × C), 137.9/138.2 (C), 140.3/140.7 (C), 172.6/173.0 (C), 174.3/175.2 (C), 199.3/200.6 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{35}H_{36}N_2O_4Na$ $[M + Na]^+$, 571.2573; found, 571.2579. Anal. Calcd for $C_{35}H_{36}N_2O_4$: C, 76.62; H, 6.61; N, 5.11. Found: C, 76.57; H, 6.84; N, 4.88.

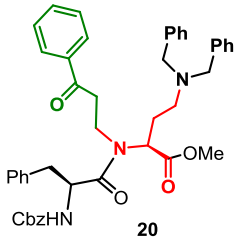
*N-(Benzoyl)-N-(3-oxo-3-phenylpropyl)-4-[O-benzyl-L-prolin-N-yl]-L-homoalanine Methyl Ester (**19**).*



19

It was obtained from the *N*-acetoxymethyl derivative (**13**) (50 mg, 0.1 mmol) using 1-phenyl-1-trimethylsiloxyethylene (96 mg, 102 μL, 0.5 mmol) according to the general addition procedure. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 70:30), yielding product (**19**) (36 mg, 65%) as a syrup. $[\alpha]_D$ −60 (*c* 1.7, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 1739, 1634, 1258, 1030 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C) rotamer mixture at 26 °C, one rotamer at 70 °C: δ_H 1.75−1.85 (2H, m), 1.91 (1H, m), 2.08 (1H, m), 2.10−2.40 (2H, m), 2.44 (1H, m), 2.56 (1H, m), 2.86 (1H, m), 3.09 (1H, m), 3.25−3.57 (2H, m), 3.67 (3H, s), 3.72 (1H, dd, *J* = 6.0, 14.8 Hz), 4.25 (1H, dd, *J* = 6.5, 14.5 Hz), 4.35−4.50 (2H, m), 5.12 (2H, s), 7.25−7.50 (12H, m), 7.58 (1H, dd, *J* = 7.3, 7.6 Hz), 7.89 (2H, br b); ^{13}C NMR (125.7 MHz, CD_3OD, 70 °C): δ_C 24.0 (CH_2), 29.4 (CH_2), 30.0 (CH_2), 38.3 (CH_2), 52.8 (CH_3), 54.0 (CH_2), 67.0 (CH_2), 67.5 (CH_2), 127.7 (CH), 129.2 (2 × CH), 129.3 (2 × CH), 129.4 (2 × CH), 129.5 (2 × CH), 129.7 (4 × CH), 130.9 (CH), 134.4 (CH), 137.5 (2 × C), 138.2 (C), 173.0 (C), 175.1 (2 × C). A (C) signal corresponding to the carbonyl group was not clearly observed. In addition, broad, very flat bands are hardly visible for CH_2 and two CH (2′-C and 2-C) signals between δ_C 48−49 and δ_C 60−65, respectively. HRMS (ESI-TOF) *m*/*z*: calcd for $C_{33}H_{36}N_2O_6Na$ $[M + Na]^+$, 579.2471; found, 579.2469. Anal. Calcd for $C_{33}H_{36}N_2O_6$: C, 71.20; H, 6.52; N, 5.03. Found: C, 71.10; H, 6.89; N, 5.10.

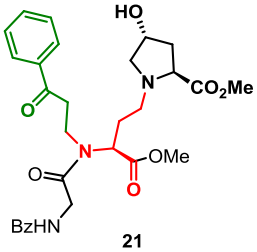
*N-(N-Benzyloxycarbonyl-L-phenylalanyl)-N-(3-oxo-3-phenylpropyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**20**).*



20

It was obtained from the *N*-acetoxymethyl derivative (**14**) (67 mg, 0.1 mmol) using 1-phenyl-1-trimethylsiloxyethylene (96 mg, 102 μL, 0.5 mmol) according to the general addition procedure. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 80:20), yielding product (**20**) (63 mg, 87%) as a syrup. $[\alpha]_D$ −19 (*c* 1.10, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3427, 3030, 1735, 1718, 1646, 1496 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C): δ_H 2.34 (1H, m), 2.82 (1H, m), 2.84−2.95 (2H, m), 3.25−3.40 (2H, m), 3.70−4.10 (11H, m), 4.52−4.62 (2H, m), 5.32 (1H, dd, *J* = 7.3, 7.3 Hz), 5.48 (1H, br d, *J* = 12.3 Hz), 5.51 (1H, br d, *J* = 12.1 Hz), 7.58−7.85 (20H, m), 7.96 (2H, dd, *J* = 7.6, 7.8 Hz), 8.05 (1H, dd, *J* = 7.2, 7.3 Hz), 8.41 (2H, br b); ^{13}C NMR (125.7 MHz, CD_3OD, 70 °C): δ_C 28.6 (CH_2), 40.2 (CH_2), 45.4 (CH_2), 51.7 (CH_2), 52.3 (CH_3), 54.0 (CH), 59.8 (2 × CH_2), 60.6 (CH), 67.8 (CH_2), 127.5 (2 × CH), 128.1 (CH), 128.8 (2 × CH), 129.0 (4 × CH), 129.2 (2 × CH), 129.5 (2 × CH), 129.6 (2 × CH), 129.8 (2 × CH), 130.3 (4 × CH), 130.6 (2 × CH), 134.5 (CH), 137.9 (C), 138.3 (C), 140.5 (C), 140.8 (2 × C), 157.9 (C), 172.8 (C), 173.9 (C), 199.7 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{45}H_{48}N_3O_6$ $[M + H]^+$, 726.3543; found, 726.3549. Anal. Calcd for $C_{45}H_{47}N_3O_6$: C, 74.46; H, 6.53; N, 5.79. Found: C, 74.21; H, 6.72; N, 5.43.

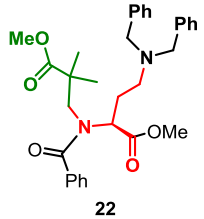
*N-(N-Benzoyl-L-glycyl)-N-(3-oxo-3-phenylpropyl)-4-(4R-hydroxy-O-Methyl)-L-homoalanine Methyl Ester (**21**).*



21

It was obtained from the *N*-acetoxymethyl derivative (**15**) (49 mg, 0.1 mmol) using 1-phenyl-1-trimethylsiloxyethylene (96 mg, 102 μL, 0.5 mmol) according to the general addition procedure but stirring for 2 h. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 20:80), yielding product (**21**) (63 mg, 84%) as a syrup. $[\alpha]_D$ −50 (*c* 0.64, MeOH); IR ($CHCl_3$) ν_{max}: 3414, 3013, 1742, 1628, 1645 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 26 °C) 2:1 rotamer mixture, minor rotamer/major rotamer: δ_H 2.00−2.20 (2H, m), ***2.23***/2.32 (2H, m), 2.52 (1H, m), 2.53 (1H, m), 2.67 (1H, m), 2.85 (1H, m), 3.02 (1H, m), 3.35−3.55 (1H, m), 3.42/3.64 (3H, s), ***3.49***/3.71 (1H, m/m), ***3.66***/3.73 (3H, s/s), 3.84 (1H, m), 4.14 (1H, m), 4.30 (1H, dd, *J* = 4.7, 8.8 Hz), ***4.39***/4.49 (1H, [d, *J* = 16.6 Hz/d, *J* = 16.8 Hz]), 4.41/***4.44*** (1H, m/m), 4.54/***4.59*** (1H, [d, *J* = 16.8 Hz/d, *J* = 17.0 Hz]), 7.42−7.65 (6H, m), 7.86 (2H, d, *J* = 7.3 Hz), ***7.97***/8.05 (2H, [d, *J* = 7.6 Hz/d, *J* = 8.5 Hz]); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 28.6/28.7 (CH_2), 40.0/40.1 (CH_2), 42.9/43.5 (CH_2), 45.0 (CH_2), 51.6 (CH_2), 52.7 (CH_3), 52.9/53.3 (CH_3), 53.5 (CH_2), 58.1/60.0 (CH), 61.8/62.4 (CH_2), 66.0/66.5 (CH), 70.6 (CH), 128.5/128.6 (2 × CH), 129.2/129.3 (2 × CH), 129.7 (2 × CH), 129.9 (2 × CH), 133.0 (CH), 134.6 (CH), 135.3/135.4 (C), 138.2/138.3 (C), 170.0/170.4 (C), 171.4 (C), 172.6/173.1 (C), 174.7/175.3 (C), 200.1 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{29}H_{35}N_3O_8Na$ $[M + Na]^+$, 576.2322; found, 576.2316. Anal. Calcd for $C_{29}H_{35}N_3O_8$: C, 62.92; H, 6.37; N, 7.59. Found: C, 62.64; H, 6.52; N, 7.67.

*(2S)-N-(3-Methoxy-2,2-dimethyl-3-oxopropyl)-N-(benzoyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**22**).*



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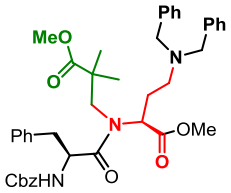
DOI: 10.1021/acs.joc.9b00114
J. Org. Chem. 2019, 84, 8392−8410

It was obtained from the *N*-acetoxymethyl derivative (**12**) (49 mg, 0.1 mmol) using as the nucleophile methyl trimethylsilyl dimethyl ketene acetal (MTDA, 87 mg, 102 μL, 0.5 mmol) according to the general addition procedure but stirring for 2 h. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 90:10), yielding product (**22**) (35 mg, 66%) as a syrup. $[\alpha]_D$ −56 (*c* 0.68, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 2970, 1735, 1636 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C) rotamer mixture at 26 °C, one rotamer at 70 °C: δ_H 1.06 (6H, br s), 2.00−2.32 (2H, m), 2.43−2.65 (2H, m), 3.45−3.70 (6H, m), 3.58 (3H, s), 3.61 (3H, s), 4.39 (1H, m), 7.15−7.45 (15H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 70 °C): δ_C 24.1 (CH_3), 25.4 (CH_3), 28.5 (CH_2), 45.3 (C), 51.3 (CH_2), 52.5 (CH_2), 52.6 (CH_2), 59.8 (CH_3), 60.1 (CH_3), 61.6 (CH), 128.2 (4 × CH), 128.5 (2 × CH), 129.4 (4 × CH), 129.7 (2 × CH), 130.5 (2 × CH), 131.2 (CH), 137.5 (C), 140.6 (C), 140.7 (C), 172.9 (C), 178.8 (2 × C); HRMS (ESI-TOF) *m*/*z*: calcd for $C_{32}H_{39}N_2O_5$ $[M + H]^+$, 531.2859; found, 531.2858. Anal. Calcd for $C_{32}H_{38}N_2O_5$: C, 72.43; H, 7.22; N, 5.28. Found: C, 72.23; H, 7.18; N, 5.55.

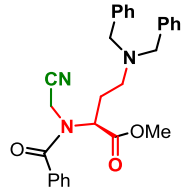
*(2S)-N-(3-Methoxy-2,2-dimethyl-3-oxopropyl)-N-(N-benzyloxycarbonyl-L-phenylalanyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**23**).*



23

It was obtained from the *N*-acetoxymethyl derivative (**14**) (67 mg, 0.1 mmol) using as the nucleophile MTDA (87 mg, 102 μL, 0.5 mmol) according to the general addition procedure but stirring for 2 h. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 80:20), yielding product (**23**) (49 mg, 69%) as a syrup. $[\alpha]_D$ −33 (*c* 0.29, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3424, 1719, 1648, 1508, 1436 cm^{-1}; ^1H NMR (500 MHz, C_6D_6, 26 °C): δ_H 1.10 (3H, s), 1.50 (3H, s), 2.07 (1H, m), 2.41−2.48 (2H, m), 2.75 (1H, m), 2.75−2.82 (2H, m), 3.10−3.40 (4H, m), 3.29 (3H, s), 3.30 (3H, s), 3.32 (1H, d, *J* = 13.3 Hz), 3.53 (1H, d, *J* = 13.3 Hz), 4.36 (1H, dd, *J* = 6.0, 6.7 Hz), 4.85 (1H, d, *J* = 12.3 Hz), 4.99 (1H, d, *J* = 12.6 Hz), 5.18 (1H, ddd, *J* = 7.0, 7.3, 8.8 Hz), 5.79 (1H, br d, *J* = 8.9 Hz), 7.00−7.25 (16H, m), 7.38 (4H, d, *J* = 7.3 Hz); ^{13}C NMR (125.7 MHz, C_6D_6, 26 °C): δ_C 22.2 (CH_3), 25.8 (CH_3), 27.7 (CH_2), 41.4 (CH_2), 45.0 (C), 51.1 (CH_2), 51.8 (CH_3), 52.1 (CH_3), 53.3 (CH), 56.7 (CH_2), 59.2 (CH_2), 60.7 (CH), 67.0 (CH_2), 127.5 (CH), 127.8 (2 × CH), 128.3 (2 × CH), 128.4 (CH), 128.5 (2 × CH), 128.6 (2 × CH), 128.7 (2 × CH), 128.9 (2 × CH), 129.0 (2 × CH), 130.0 (2 × CH), 130.4 (2 × CH), 137.2 (C), 137.7 (C), 140.1 (2 × C), 156.0 (C), 171.4 (C), 172.9 (C), 177.2 (C); HRMS (ESI-TOF) *m*/*z*: calcd for $C_{42}H_{50}N_3O_7$ $[M + H]^+$, 708.3649; found, 708.3649. Anal. Calcd for $C_{42}H_{49}N_3O_7$: C, 71.26; H, 6.98; N, 5.94. Found: C, 71.48; H, 7.22; N, 6.17.

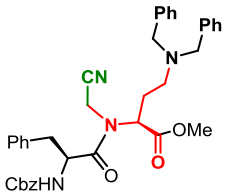
*(2S)-N-(Cyanomethyl)-N-(benzoyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**24**).* It was obtained from the *N*-acetoxymethyl derivative (**12**) (49 mg, 0.1 mmol) using as the nucleophile methyl trimethylsilyl cyanide (50 mg, 63 μL, 0.5 mmol)according to the general addition procedure but stirring for 2 h. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 80:20), yielding product (**24**) (36 mg, 79%) as a syrup. Rotamer mixture at 26 °C, one rotamer at 70 °C. $[\alpha]_D$ −8



24

(*c* 0.36, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 2980, 1741, 1655, 1424 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C): δ_H 1.98 (1H, m), 2.20 (1H, m), 2.50−2.55 (2H, m), 3.47 (2H, br d, *J* = 12.0 Hz), 3.55 (2H, br d, *J* = 13.3 Hz), 3.64 (3H, s), 4.23 (2H, br s), 4.71 (1H, m), 7.15−7.22 (6H, m), 7.23 (2H, d, *J* = 7.3 Hz), 7.25 (2H, dd, *J* = 6.9, 7.3 Hz), 7.34 (2H, d, *J* = 7.3 Hz), 7.45 (2H, dd, *J* = 7.3, 7.9 Hz), 7.52 (1H, dd, *J* = 7.2, 7.6 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 70 °C): δ_C 28.9 (CH_2), 51.3 (CH_2), 53.1 (CH_3), 59.8 (2 × CH_2), 60.0 (CH), 117.2 (C), 128.1 (2 × CH), 128.2 (2 × CH), 129.5 (4 × CH), 130.1 (2 × CH), 130.3 (4 × CH), 132.0 (CH), 135.7 (C), 140.5 (2 × C), 172.3 (C), 174.5 (C); HRMS (ESI-TOF) *m*/*z*: calcd for $C_{28}H_{30}N_3O_3$ $[M + H]^+$, 456.2287; found, 456.2285. Anal. Calcd for $C_{28}H_{29}N_3O_3$: C, 73.82; H, 6.42; N, 9.22. Found: C, 73.60; H, 6.57; N, 9.03.

*(2S)-N-(Cyanomethyl)-N-(N-benzyloxycarbonyl-L-phenylalanyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**25**).*



25

It was obtained from the *N*-acetoxymethyl derivative (**14**) (67 mg, 0.1 mmol) using as the nucleophile methyl trimethylsilyl cyanide (50 mg, 63 μL, 0.5 mmol) according to the general addition procedure but stirring for 2 h. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 80:20), yielding product (**25**) (49 mg, 78%) as a syrup. Rotamer mixture at 26 and 70 °C, one rotamer at 100 °C. $[\alpha]_D$ −7 (*c* 0.43, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3429, 1741, 1716, 1670, 1507, 1455 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C) rotamer mixture, one major rotamer: δ_H 1.70 (1H, m), 2.17 (1H, m), 2.30−2.50 (2H, m), 2.88 (1H, dd, *J* = 7.6, 13.8 Hz), 3.04 (1H, m), 3.45−3.60 (4H, m), 3.53 (3H, s), 4.01 (1H, d, *J* = 17.1 Hz), 4.19 (1H, d, *J* = 17.4 Hz), 4.65−4.90 (2H, m), 4.97 (1H, d, *J* = 12.3 Hz), 5.01 (1H, d, *J* = 12.9 Hz), 7.05−7.40 (20H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 70 °C) some signals are still split due to the rotamer mixture: δ_C 27.2/29.1 (CH_2), 32.5/36.4 (CH_2), 39.2/39.9 (CH_2), 51.5 (CH_2), 53.1 (CH_3), 54.1 (CH), 59.2 (CH), 59.8 (2 × CH_2), 68.0 (CH_2), 117.0 (C), 128.1 (CH), 128.2 (2 × CH), 129.3 (2 × CH), 129.5 (CH), 129.6 (4 × CH), 129.7 (2 × CH), 130.4 (2 × CH), 130.7 (4 × CH), 130.8 (2 × CH), 137.9 (2 × C), 140.4 (2 × CH), 157.6 (C), 172.1 (C), 174.3 (C); ^1H NMR (500 MHz, DMSO-d_6, 100 °C) one visible rotamer: δ_H 1.85 (1H, m), 2.16 (1H, m), 2.30−2.50 (2H, m), 2.87 (1H, dd, *J* = 8.5, 13.9 Hz), 2.96 (1H, dd, *J* = 3.5, 13.9 Hz), 3.45−3.60 (4H, m), 3.53 (3H, s), 4.00−4.50 (2H, m), 4.68 (1H, ddd, *J* = 6.0, 8.5, 8.5 Hz), 4.77 (1H, m), 4.92 (1H, d, *J* = 12.9 Hz), 4.97 (1H, d, *J* = 12.6 Hz), 7.10−7.45 (20H, m); ^{13}C NMR (125.7 MHz, DMSO-d_6, 100 °C): δ_C 26.4 (CH_2), 31.4 (CH_2), 37.2 (CH_2), 49.6 (CH_2), 51.4 (CH_3), 52.1 (CH), 56.6 (CH), 57.1 (2 × CH_2), 65.3 (CH_2), 115.8 (C), 126.0 (CH), 126.3 (CH), 126.9 (2 × CH), 127.2 (2 × CH), 127.6 (2 × CH), 127.8 (4 × CH), 128.1 (2 × CH), 128.2 (4 × CH), 128.8 (2 × CH), 136.6 (2 × C),

DOI: 10.1021/acs.joc.9b00114
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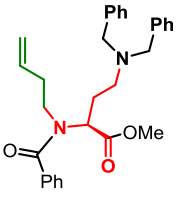
138.7 (2 × C), 155.3 (C), 169.8 (C), 171.6 (C); HRMS (ESI-TOF) m/z: calcd for $C_{38}H_{41}N_4O_5$ $[M + H]^+$, 633.3077; found, 633.3077. Anal. Calcd for $C_{38}H_{40}N_4O_5$: C, 72.13; H, 6.37; N, 8.85. Found: C, 71.97; H, 6.31; N, 8.76.

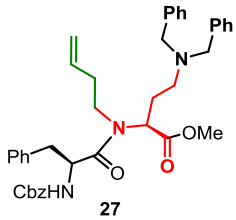
*(2S)-N-(But-3-en-1-yl)-N-(benzoyl)-4-dibenzylamino-L-homoalanine Methyl Ester (**26**).*



26

It was obtained from the *N*-acetoxymethyl derivative (**12**) (49 mg, 0.1 mmol) using as the nucleophile allyltrimethylsilane (57 mg, 80 μL, 0.5 mmol) according to the general addition procedure but stirring for 1.5 h. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 80:20), yielding product (**26**) (39 mg, 82%) as a colorless oil. $[\alpha]_D$ −55 (*c* 0.39, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3069, 1737, 1633, 1495 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C) rotamer mixture at 26 °C, one rotamer at 70 °C: δ_H 1.96 (1H, m), 2.20 (2H, m), 2.37 (1H, m), 2.56 (2H, m), 3.05−3.30 (2H, m), 3.35−3.62 (4H, m), 3.63 (3H, s), 4.30 (1H, m), 4.94 (2H, br d, J = 9.5 Hz), 5.56 (1H, m), 7.15−7.50 (15H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 70 °C): δ_C 28.9 (CH_2), 34.1 (CH_2), 51.4 (CH_2), 51.5 (CH_2), 52.6 (CH_3), 59.7 (CH), 59.9 (2 × CH_2), 117.2 (CH_2), 127.6 (CH), 128.1 (3 × CH), 129.5 (4 × CH), 129.7 (2 × CH), 130.2 (4 × CH), 130.9 (CH), 135.9 (CH), 137.6 (C), 140.8 (2 × C), 172.7 (C), 174.3 (C). HRMS (ESI-TOF) m/z: calcd for $C_{30}H_{34}N_2O_3Na$ $[M + Na]^+$, 493.2467; found, 493.2468. Anal. Calcd for $C_{30}H_{34}N_2O_3$: C, 76.57; H, 7.28; N, 5.95. Found: C, 76.20; H, 7.38; N, 6.06.

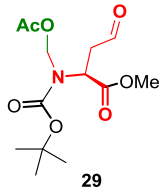
*(2S)-N-(But-3-en-1-yl)-N-[N-(benzyloxycarbonyl)-phenylalanyl]-4-dibenzylamino-L-homoalanine Methyl Ester (**27**).*



27

It was obtained from the *N*-acetoxymethyl derivative (**14**) (67 mg, 0.1 mmol) using as the nucleophile allyltrimethylsilane (57 mg, 80 μL, 0.5 mmol) according to the general addition procedure but stirring for 1.5 h. After work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 80:20), yielding product (**27**) (52 mg, 80%) as a colorless oil. $[\alpha]_D$ −17 (*c* 0.39, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3427, 1734, 1716, 1645, 1507, 1496, 1455 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 70 °C) rotamer mixture at 26 °C, one rotamer at 70 °C: δ_H 1.69 (1H, m), 2.09 (1H, m), 2.21 (1H, m), 2.32 (1H, m), 2.40−2.55 (2H, m), 2.79 (1H, dd, J = 7.2, 12.9 Hz), 2.86 (1H, dd, J = 7.4, 13.4 Hz), 2.95 (1H, m), 3.11 (1H, m), 3.50 (3H, s), 3.53 (2H, d, J = 14.2 Hz), 3.58 (2H, d, J = 13.3 Hz), 3.97 (1H, dd, J = 6.5, 6.8 Hz), 4.69 (1H, m), 4.93−5.10 (4H, m), 5.67 (1H, m), 7.14−7.31 (20H, m); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 28.7 (CH_2), 34.3 (CH_2), 40.3 (CH_2), 49.9 (CH_2), 51.7 (CH_2), 52.5 (CH_3), 59.6 (CH), 59.8 (2 × CH_2), 59.9 (CH), 67.9 (CH_2), 117.7 (CH_2), 128.0 (CH), 128.1 (4 × CH), 129.0 (CH), 129.3 (4 × CH), 129.5 (2 × CH), 129.6 (2 × CH), 130.3 (4 × CH), 130.6 (2 × CH), 135.6 (CH), 137.9 (2 × C), 140.7 (2 × C), 172.8 (C), 173.4 (C). A (C) signal corresponding to carbamate carbonyl was not observed. HRMS (ESI-TOF) m/z: calcd for $C_{40}H_{45}N_3O_5Na$ $[M + Na]^+$, 670.3257; found, 670.3257. Anal. Calcd for $C_{40}H_{45}N_3O_5$: C, 74.16; H, 7.00; N, 6.49. Found: C, 74.03; H, 7.13; N, 6.27.

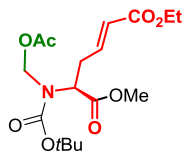
*(2S)-N-(Acetoxymethyl)-N-(tert-butoxycarbonyl)-4-oxo-L-homoalanine Methyl Ester (**29**).*



29

It was obtained from *N*-(*tert*-butoxycarbonyl)-L-hydroxyproline methyl ester (**28**) (245.13 mg, 1.0 mmol) extrapolating the general scission procedure. After purification by rotatory chromatography (hexanes/EtOAc 90:10), homoalanine derivative **29** was obtained (253 mg, 83%) as a yellowish oil: $[\alpha]_D$ −81 (*c* 0.52, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3028, 1739, 1416, 1369, 1254 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 70 °C) rotamer mixture at 26 °C, one visible rotamer at 70 °C: δ_H 1.46 (9H, s), 2.04 (3H, s/s), 2.94 (1H, m), 3.30 (1H, dd, J = 6.1, 18 Hz), 3.72 (3H, s), 4.82 (1H, m), 5.41 (2H, br s), 9.77 (1H, s); ^{13}C NMR (125.7 MHz, $CDCl_3$, 70 °C): δ_C 20.7 (CH_3), 28.2 (3 × CH_3), 45.2 (CH_2), 52.4 (CH_3), 55.5 (CH), 72.9 (CH_2), 82.4 (C), 154.0 (C), 170.5 (2 × C), 198.1 (C). HRMS (ESI-TOF): calcd for $C_{14}H_{25}NO_8Na$ (M^+ + Na + MeOH), 358.1478; found, 358.1477. Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.73; H, 7.12; N, 4.46.

*1-O-Methyl 5-O-Ethyl N-(Acetoxymethyl)-N-(tert-butoxycarbonyl)-4,5-didehydro-L-homoglutamate (**30**).*



30

It was obtained from scission product **29** (160.0 mg, 0.53 mmol) using a HWE reaction. The HWE reagent was prepared from triethyl phosphonoacetate (117 μL, 132 mg, 0.58 mmol) which was slowly added to a suspension of sodium hydride (60% in mineral oil, 24 mg, 0.58 mmol) in dry THF (3 mL) at −20 °C and stirred for 1 h.

Then, a solution of crude aldehyde **29** in dry THF (2 mL) was added dropwise to the HWE reagent, and the mixture was stirred at −20 °C for 2 h before being poured into water and extracted with diethyl ether. The organic layer was dried, filtered, and evaporated as usual, and the residue was purified by rotatory chromatography (hexanes/EtOAc 90:10), affording derivative **30** (183.4 mg, 93%) as a yellowish oil: $[\alpha]_D$ −46 (*c* 0.39, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3024, 1742, 1713, 1659, 1438, 1370, 1260 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C) rotamer mixture at 26 °C: δ_H 1.27 (3H, dd, J = 7.1, 7.1 Hz), 1.45/1.47 (9H, s/s), 2.07 (3H, s), 2.74−2.90 (1H, m), 2.89−2.96 (1H, m), 3.71 (3H, s), 4.18 (2H, ddd, J = 7.1, 7.1, 7.1 Hz), 4.34/4.49 (1H, m/m), 5.25 (1H*min*, d, J = 11.2 Hz), 5.36 (2H*major*, br s), 5.37 (1H*min*, d, J = 11.0 Hz), 5.88 (1H, d, J = 15.5 Hz), 6.89 (1H, ddd, J = 6.8, 8.5, 15.2 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 14.2 (CH_3), 20.8 (CH_3), 28.1 (3 × CH_3), 32.4/33.4 (CH_2), 52.5 (CH_3), 59.0/60.2 (CH), 60.3 (CH_2), 71.7/72.6 (CH_2), 82.1/82.5 (C), 124.2/124.3 (CH), 143.8 (CH), 154.1 (C), 165.9 (C), 170.6 (C), 170.7/171.4 (C). HRMS (ESI-TOF): calcd for $C_{17}H_{27}NO_8Na$ (M^+ + Na), 396.1634; found, 396.1631. Anal.

DOI: 10.1021/acs.joc.9b00114
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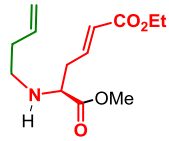
Calcd for $C_{17}H_{27}NO_8$: C, 54.68; H, 7.29; N, 3.75. Found: C, 54.50; H, 7.06; N, 3.40.

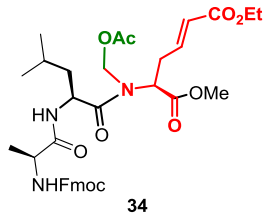
*1-O-Methyl 5-O-Ethyl N-(But-3-en-1-yl)-N-(tert-butoxycarbonyl)-4,5-didehydro-L-homoglutamate (**31**).*



31

It was obtained from compound **30** (37.3 mg, 0.1 mmol). Because of the lability of the Boc group under acid conditions, the general protocol for nucleophilic addition afforded a complex mixture of products. Therefore, the general protocol was modified, and the best results were obtained by using a more dilute solution (MeCN, 4 mL) and a lower temperature (−40 °C) and stirring for 3 h. After usual work-up and purification by rotatory chromatography (hexanes/EtOAc 90:10), derivative **31** was obtained (19.6 mg, 77%) as a yellowish oil: $[\alpha]_D$ +15 (*c* 0.38, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3030, 1731, 1716, 1656, 1370, 1266 cm^{-1}; ^1H NMR (500 MHz, CD_3OD, 26 °C): δ_H 1.26 (3H, dd, *J* = 7.1, 7.3 Hz), 2.23 (2H, m), 2.53−2.57 (3H, m), 2.60 (1H, m), 3.47 (1H, dd, *J* = 6.5, 6.6 Hz), 3.71 (3H, s), 4.16 (2H, ddd, *J* = 7.1, 7.1, 7.3 Hz), 5.02 (1H, br d, *J* = 10.2 Hz), 5.09 (1H, br d, *J* = 17.1 Hz), 5.78 (1H, m), 5.89 (1H, ddd, *J* = 1.3, 1.5, 15.6 Hz), 6.88 (1H, ddd, *J* = 7.4, 7.8, 15.6 Hz); ^{13}C NMR (125.7 MHz, CD_3OD, 26 °C): δ_C 14.5 (CH_3), 34.9 (CH_2), 36.3 (CH_2), 48.5 (CH_2), 52.4 (CH_3), 61.3 (CH), 61.5 (CH_2), 116.9 (CH_2), 125.0 (CH), 137.2 (CH), 145.3 (CH), 167.7 (C), 175.3 (C). HRMS (ESI-TOF): calcd for $C_{13}H_{21}NO_4Na$ (M^+ + Na), 278.1368; found, 278.1368.

*1-O-Methyl 5-O-Ethyl N-(Acetoxymethyl)-N-(fluorenylmethyloxycarbonyl-L-alanyl-L-leucyl)-4,5-didehydro-L-homoglutamate (**34**).*



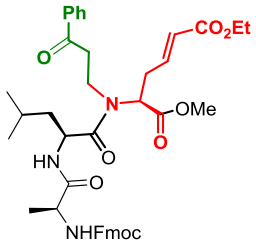
34

To a solution of Fmoc-Ala-Leu-Hyp-OMe (**32**) (110 mg, 0.2 mmol) in dry dichloroethane (4 mL) was added iodine (76 mg, 0.3 mmol) and DIB (258 mg, 0.8 mmol). The reaction mixture was stirred at reflux for 20 min under irradiation with visible light (80 W tungsten lamp). Then, it was poured into 10% aqueous $Na_2S_2O_3$ and extracted with CH_2Cl_2. After drying over sodium sulfate, the organic layer was filtered and evaporated under vacuum to give a residue containing the scission product (aldehyde **33**), which was not purified but was used directly in a HWE reaction.

The HWE reagent was prepared from triethyl phosphonoacetate (44 μL, 49.7 mg, 0.22 mmol) which was slowly added to a suspension of sodium hydride (60% in mineral oil, 9 mg, 0.22 mmol) in dry THF (3 mL) at −20 °C and stirred for 1 h.

Then, a solution of crude aldehyde **33** in dry THF (2 mL) was added dropwise to the HWE reagent, and the mixture was stirred at −20 °C for 2 h before being poured into water and extracted with diethyl ether. The organic layer was dried, filtered, and evaporated as usual, and the residue was purified by rotatory chromatography (hexanes/EtOAc, 60:40), yielding product (**34**) (69 mg, 51%) as a syrup. $[\alpha]_D$ −53 (*c* 0.29, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3422, 1746, 1716, 1671, 1506, 1449 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 0.90 (3H, d, *J* = 6.6 Hz), 0.95 (3H, d, *J* = 6.3 Hz), 1.26 (3H, dd, *J* = 7.0, 7.3 Hz), 1.37 (3H, br d, *J* = 5.5 Hz), 1.50−1.58 (2H, m), 1.59 (1H, m), 2.11 (3H, s), 2.85−3.00 (2H, m), 3.68 (3H, s), 4.16 (2H, ddd, *J* = 7.2, 7.3, 7.3 Hz), 4.20−4.28 (2H, m), 4.35−4.44 (2H, m), 4.55 (1H, br dd, *J* = 6.7, 8.5 Hz), 5.01−5.10 (1H, m), 5.38 (1H, br b), 5.43 (1H, d, *J* = 12.6 Hz), 5.60 (1H, d, *J* = 12 Hz), 5.83 (1H, d, *J* = 16 Hz), 6.34 (1H, br d, *J* = 5.4 Hz), 6.78 (1H, m), 7.31 (2H, dd, *J* = 7.3, 7.6 Hz), 7.40 (2H, dd, *J* = 7.3, 7.6 Hz), 7.59 (2H, d, *J* = 7.3 Hz), 7.77 (2H, d, *J* = 7.6 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 14.2 (CH_3), 18.7 (CH_3), 20.6 (CH_3), 21.7 (CH_3), 23.0 (CH_3), 24.7 (CH), 31.4 (CH_2), 42.3 (CH_2), 47.0 (CH), 48.1 (CH), 50.2 (CH), 52.5 (CH_3), 59.5 (CH), 60.3 (CH_2), 67.0 (CH_2), 71.7 (CH_2), 119.9 (2 × CH), 124.3 (CH), 125.0 (2 × CH), 127.0 (2 × CH), 127.6 (2 × CH), 141.2 (2 × C), 143.4 (CH), 143.7 (C), 143.8 (C), 155.8 (C), 165.8 (C), 169.7 (C), 170.5 (C), 172.0 (C), 174.4 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{36}H_{45}N_3O_{10}$ $[M]^+$, 679.3105; found, 679.3085. Anal. Calcd for $C_{36}H_{45}N_3O_{10}$: C, 63.61; H, 6.67; N, 6.18. Found: C, 63.24; H, 7.06; N, 5.89.

*1-O-Methyl 5-O-Ethyl N-(3-Oxo-3-phenylpropyl)-N-(fluorenylmethyloxycarbonyl-L-alanyl-L-leucyl)-4,5-didehydro-L-homoglutamate (**35**).*

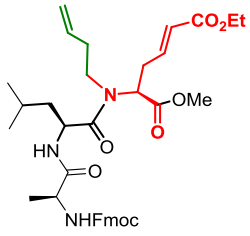


35

It was obtained from the *N*-acetoxymethyl derivative (**34**) (68 mg, 0.1 mmol) according to the general addition procedure using 1-phenyl-1-trimethylsiloxyethylene (96 mg, 102 μL, 0.5 mmol) as the nucleophile and stirring for 5 h. After usual work-up and solvent evaporation, the residue was purified by rotatory chromatography (hexanes/EtOAc, 70:30), yielding product (**35**) (54 mg, 73%) as a syrup. $[\alpha]_D$ −70 (*c* 0.40, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3421, 1741, 1712, 1683, 1652, 1506 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 0.91 (3H, d, *J* = 6.7 Hz), 0.95 (3H, d, *J* = 6.7 Hz), 1.26 (3H, dd, *J* = 7.0, 7.2 Hz), 1.37 (3H, br s), 1.43 (1H, m), 1.55 (1H, m), 1.68 (1H, m), 2.90−2.95 (2H, m), 3.43 (1H, m), 3.46 (3H, s), 3.58 (1H, m), 3.77 (1H, m), 3.88 (1H, m), 4.12−4.33 (5H, m), 4.37 (2H, br d, *J* = 6.6 Hz), 4.97 (1H, m), 5.45 (1H, br d, *J* = 7.0 Hz), 5.86 (1H, d, *J* = 15.5 Hz), 6.49 (1H, br b), 6.89 (1H, m), 7.30 (2H, dd, *J* = 7.3, 7.6 Hz), 7.39 (2H, dd, *J* = 7.3, 7.6 Hz), 7.48 (2H, m), 7.57 (1H, m), 7.58 (2H, d, *J* = 7.3 Hz), 7.75 (2H, d, *J* = 7.6 Hz), 7.99 (2H, d, *J* = 7.3 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 14.2 (CH_3), 18.8 (CH_3), 21.6 (CH_3), 23.2 (CH_3), 24.7 (CH), 31.7 (CH_2), 38.3 (CH_2), 42.3 (CH_2), 44.5 (CH_2), 47.1 (CH), 47.9 (CH), 50.4 (CH), 52.2 (CH_3), 60.4 (CH_2), 60.8 (CH), 67.0 (CH_2), 119.9 (2 × CH), 124.6 (CH), 125.0 (CH), 125.4 (CH), 127.0 (CH), 127.7 (CH), 128.0 (CH), 128.1 (CH), 128.6 (2 × CH), 128.7 (2 × CH), 133.5 (CH), 136.3 (2 × C), 141.3 (3 × C), 143.8 (CH), 155.7 (C), 165.9 (C), 169.9 (C), 172.2 (C), 172.8 (C), 197.7 (C). HRMS (ESI-TOF) *m*/*z*: calcd for $C_{42}H_{49}N_3O_9$ $[M]^+$, 739.3469; found, 739.3489. Anal. Calcd for $C_{42}H_{49}N_3O_9$: C, 68.18; H, 6.68; N, 5.68. Found: C, 67.88; H, 7.06; N, 5.55.

*1-O-Methyl 5-O-Ethyl N-(But-3-en-1-yl)-N-(fluorenylmethyloxycarbonyl-L-alanyl-L-leucyl)-4,5-didehydro-L-homoglutamate (**36**).* It was obtained from the *N*-acetoxymethyl derivative (**34**) (68 mg, 0.1 mmol) according to the general addition procedure using allyltrimethylsilane (57 mg, 80 μL, 0.5 mmol) as the nucleophile and stirring for 4 h. After usual work-up and solvent evaporation, the residue

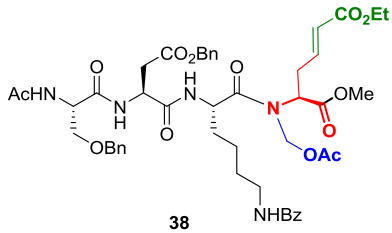
DOI: 10.1021/acs.joc.9b00114
J. Org. Chem. 2019, 84, 8392−8410



36

was purified by rotatory chromatography (hexanes/EtOAc, 70:30), yielding product (**36**) (40 mg, 61%) as a syrup. $[\alpha]_D$ −73 (*c* 0.57, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3421, 1717, 1682, 1651, 1506 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 0.88 (3H, d, *J* = 6.9 Hz), 0.96 (3H, d, *J* = 6.6 Hz), 1.27 (3H, dd, *J* = 6.9, 7.3 Hz), 1.37 (3H, br s), 1.38 (1H, m), 1.57 (1H, m), 1.65 (1H, m), 2.40 (1H, m), 2.49 (1H, m), 2.85 (1H, m), 2.96 (1H, m), 3.27 (1H, m), 3.44 (1H, m), 3.69 (3H, s), 4.12−4.35 (5H, m), 4.36−4.45 (2H, m), 4.93 (1H, m), 5.12 (1H, d, *J* = 10.1 Hz), 5.18 (1H, d, *J* = 17.4 Hz), 5.44 (1H, br b), 5.79 (1H, m), 5.87 (1H, d, *J* = 15.8 Hz), 6.45 (1H, m), 6.86 (1H, m), 7.31 (2H, dd, *J* = 7.3, 7.6 Hz), 7.39 (2H, dd, *J* = 7.3, 7.6 Hz), 7.59 (2H, d, *J* = 7.3 Hz), 7.75 (2H, d, *J* = 7.6 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 14.2 (CH_3), 18.9 (CH_3), 21.6 (CH_3), 23.3 (CH_3), 24.7 (CH), 31.8 (CH_2), 33.6 (CH_2), 42.7 (CH_2), 47.1 (CH), 47.8 (CH), 49.0 (CH_2), 50.4 (CH), 52.4 (CH_3), 59.5 (CH), 60.4 (CH_2), 67.0 (CH_2), 118.1 (CH_2), 119.9 (2 × CH), 124.5 (CH), 125.1 (2 × CH), 127.1 (2 × CH), 127.7 (2 × CH), 133.5 (CH), 141.3 (2 × C), 143.6 (C), 143.8 (C), 143.9 (CH), 155.7 (C), 165.9 (C), 170.0 (C), 171.9 (C), 172.7 (C). HRMS (ESI-TOF) *m/z*: calcd for $C_{37}H_{47}N_3O_8$ $[M]^+$, 661.3363; found, 661.3345. Anal. Calcd for $C_{37}H_{47}N_3O_8$: C, 67.15; H, 7.16; N, 6.35. Found: C, 66.85; H, 7.55; N, 6.32.

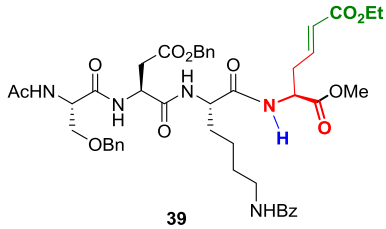
*1-O-Methyl 5-O-Ethyl N-[N²-(N-Acetyl-O-benzyl-L-seryl-O-benzyl-L-asparty)-N⁶-(benzoyl)-L-lysyl]-N-acetoxymethyl-4,5-didehydro-L-homoglutamate (**38**).*



38

It was obtained from substrate **37** (460 mg, 0.574 mmol) according to the scission and subsequent HWE protocol commented for compound **31**. The scission, however, was carried out at 89 °C for 4 h, and the HWE was performed at −20 °C for 2 h. After purification by rotatory chromatography (CH_2Cl_2/MeOH 97:3), peptide **38** was obtained (352 mg, 66%) as a yellowish oil: $[\alpha]_D$ −19 (*c* 0.97, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3417, 3030, 1743, 1664, 1525, 1235, 1193 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 26 °C): δ_H 1.26 (3H, dd, *J* = 6.8, 6.9 Hz), 1.30−1.75 (6H, m), 1.98 (3H, s), 2.07 (3H, s), 2.70 (1H, dd, *J* = 5.2, 17.2 Hz), 2.85−2.98 (2H, m), 3.06 (1H, dd, *J* = 5.1, 17.2 Hz), 3.30−3.53 (2H, m), 3.59 (1H, dd, *J* = 6.2, 9.3 Hz), 3.67 (3H, s), 3.84 (1H, dd, *J* = 4.4, 9.6 Hz), 4.16 (2H, ddd, *J* = 6.8, 7.2, 7.2 Hz), 4.48−4.65 (4H, m), 4.83 (1H, ddd, *J* = 4.6, 5.2, 8.7 Hz), 5.02 (1H, m), 5.03 (1H, d, *J* = 12.1 Hz), 5.06 (1H, d, *J* = 12.5 Hz), 5.35 (1H, d, *J* = 12.7 Hz), 5.60 (1H, d, *J* = 12.4 Hz), 5.82 (1H, d, *J* = 15.6 Hz), 6.59 (1H, d, *J* = 6.4 Hz), 6.79 (1H, ddd, *J* = 7.4, 7.9, 15.6 Hz), 6.95 (1H, m), 7.20−7.50 (14H, m), 7.63 (1H, d, *J* = 8.7 Hz), 7.81 (2H, *J* = 7.3 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, 26 °C): δ_C 14.2 (CH_3), 20.6 (CH_3), 22.5 (CH_2), 22.9 (CH_3), 28.7 (CH_2), 31.4 (CH_2), 32.3 (CH_2), 35.2 (CH_2), 39.5 (CH_2), 49.3 (CH), 49.5 (CH), 52.6 (CH_3), 53.3 (CH), 59.2 (CH), 60.4 (CH_2), 66.8 (CH_2), 69.1 (CH_2), 71.4 (CH_2), 73.4 (CH_2), 124.3 (CH), 127.0 (2 × CH), 127.7 (2 × CH), 128.0 (CH), 128.1 (2 × CH), 128.3 (4 × CH), 128.5 (4 × CH), 131.2 (CH), 134.7 (C), 135.2 (C), 137.2 (C), 143.5 (CH), 166.0 (C), 167.7 (C), 169.7 (C), 169.8 (C), 169.9 (C), 170.6 (C), 170.7 (C), 171.6 (C), 173.3 (C). HRMS (ESI-TOF): calcd for $C_{48}H_{59}N_5O_{14}Na$ (M^+ + Na), 952.3956; found, 952.3958. Anal. Calcd for $C_{48}H_{59}N_5O_{14}$: C, 61.99; H, 6.39; N, 7.53. Found: C, 62.01; H, 6.58; N, 7.51.

*1-O-Methyl 5-O-Ethyl N-[N²-(N-Acetyl-O-benzyl-L-seryl-O-benzyl-L-asparty)-N⁶-(benzoyl)-L-lysyl]-4,5-didehydro-L-homoglutamate (**39**).*



39

It was obtained from peptide **38** (46.5 mg, 0.05 mmol) by performing the usual addition protocol but instead of adding a nucleophile, the reaction mixture was poured into cold aqueous $NaHCO_3$ solution. After usual work-up and purification by rotatory chromatography (CH_2Cl_2/MeOH 97:3), derivative **39** was obtained (32 mg, 76%) as a yellowish oil: $[\alpha]_D$ +6 (*c* 0.54, $CHCl_3$); IR ($CHCl_3$) ν_{max}: 3418, 3344, 3068, 1717, 1663, 1453, 1427, 1209 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 26 °C): δ_H 1.24 (3H, dd, *J* = 7.0, 7.1 Hz), 1.35−1.42 (2H, m), 1.52−1.62 (2H, m), 1.69 (1H, m), 1.90 (1H, m), 1.98 (3H, s), 2.64 (1H, ddd, *J* = 7.3, 7.4, 14.7 Hz), 2.72 (1H, m), 2.75 (1H, dd, *J* = 5.6, 17.2 Hz), 3.13 (1H, dd, *J* = 4.8, 17.2 Hz), 3.35−3.45 (2H, m), 3.63 (1H, dd, *J* = 5.8, 9.8 Hz), 3.71 (3H, s), 3.82 (1H, dd, *J* = 4.6, 10 Hz), 4.13 (2H, ddd, *J* = 7.0, 7.1, 7.5 Hz), 4.38 (1H, dd, *J* = 4.3, 9.0 Hz), 4.45 (1H, dd, *J* = 4.6, 5.4 Hz), 4.52 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.65 (1H, dd, *J* = 5.2, 7.1 Hz), 4.78 (1H, m), 5.02 (1H, d, *J* = 12.5 Hz), 5.07 (1H, d, *J* = 12.3 Hz), 5.88 (1H, d, *J* = 15.6 Hz), 6.84 (1H, ddd, *J* = 7.3, 7.9, 15.2 Hz), 7.25−7.40 (16H, m), 7.44 (1H, dd, *J* = 7.3, 7.5 Hz), 7.57 (1H, d, *J* = 8.2 Hz), 7.76 (2H, d, *J* = 7.3 Hz). Two NH signals appear as broad bands overlapped in the aromatic region and are not readily integrated; ^{13}C NMR (125.7 MHz, $CDCl_3$, 26 °C): δ_C 14.2 (CH_3), 22.7 (CH_3), 22.9 (CH_2), 28.7 (CH_2), 30.5 (CH_2), 34.4 (CH_2), 34.9 (CH_2), 39.3 (CH), 49.9 (CH_3), 51.2 (CH), 52.6 (CH), 53.3 (CH), 54.0 (CH_2), 60.4 (CH_2), 66.9 (CH_2), 68.8 (CH_2), 73.5 (CH_2), 124.8 (CH), 127.0 (2 × CH), 127.8 (2 × CH), 128.1 (2 × CH), 128.2 (CH), 128.4 (3 × CH), 128.6 (4 × CH), 131.3 (CH), 134.7 (C), 135.1 (C), 137.0 (C), 142.6 (CH), 166.1 (C), 167.6 (C), 170.1 (C), 170.2 (C), 170.3 (C), 171.1 (C), 171.3 (C), 171.8 (C); HRMS (ESI-TOF): calcd for $C_{45}H_{55}N_5O_{12}Na$ (M^+ + Na), 880.3745; found, 880.3750. Anal. Calcd for $C_{45}H_{55}N_5O_{12}$: C, 63.00; H, 6.46; N, 8.16. Found: C, 63.25; H, 6.75; N, 7.94.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.9b00114.

General scheme for the preparation of the scission substrates **32** and **37**, reproductions of ^1H and ^{13}C NMR spectra of compounds **8**, **9**, **11**−**13**, **11**−**25**, **29**−**32**, **34**−**39**, synthetic intermediates for the scission substrates **40** and **41**−**45**, and the HSQC experiment for compound **39** (PDF)

DOI: 10.1021/acs.joc.9b00114
J. Org. Chem. 2019, 84, 8392−8410

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Research Program SAF-2013-48399-R, Plan Estatal de I+D, Ministerio de Economía, Industria, y Competitividad, Spain (currently Ministerio de Ciencia, Innovación y Universidades) and European Social Funds (FSE). D.H. thanks her current contract financed by Cabildo de Tenerife, included in Tenerife 2030 under the Program TF INNOVA 2016-21 (with MEDI & FDCAN Funds). C.J.S. was the recipient of a postdoctoral contract from the Torres Quevedo Programme (PTQ15-07923), and C.C. was granted a contract from the Doctorados Industriales Programme (DI-14-06736). Both industrial Programmes were sponsored by MINECO-Ministerio de Ciencia, Innovación y Universidades, and cofinanced by the company BIOSIGMA SL (Spain). C.C. is currently a student of the Ph.D. Program "Química e Ingeniería Química" of the University of La Laguna (ULL). We also thank CSIC (Spanish Research Council) for partially funding green Open Access to this article.

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