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# Overcoming Chemical Challenges in the Solid-Phase Synthesis of High-Purity GnRH Antagonist Degarelix. Part 2

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**ABSTRACT:** The hydrolysis and rearrangement of the dihydroorotic (Hor) residue in the presence of bases, leading to the formation of the hydantoin (Hyd) impurity, represent one of the major problems in manufacturing of the gonadotropin-releasing hormone antagonist Degarelix. In an attempt to find efficient strategies to overcome this problem, we carried out a screening of organic bases in order to select those which afforded both the rapid Fmoc deprotection during the solid-phase synthesis and the absence of this peculiar rearrangement. Among the bases tested, only *tert*-butylamine did not affect the peptide molecule and was able to perform fast Fmoc removal. The use of *tert*-butylamine for the synthesis of Degarelix led to a product with excellent purity and yield without a detectable amount of the hydantoin impurity. Thus, we showed that *tert*-butylamine can be a suitable alternative to piperidine for industrial-scale production of Degarelix or other Hor-containing peptide pharmaceuticals.

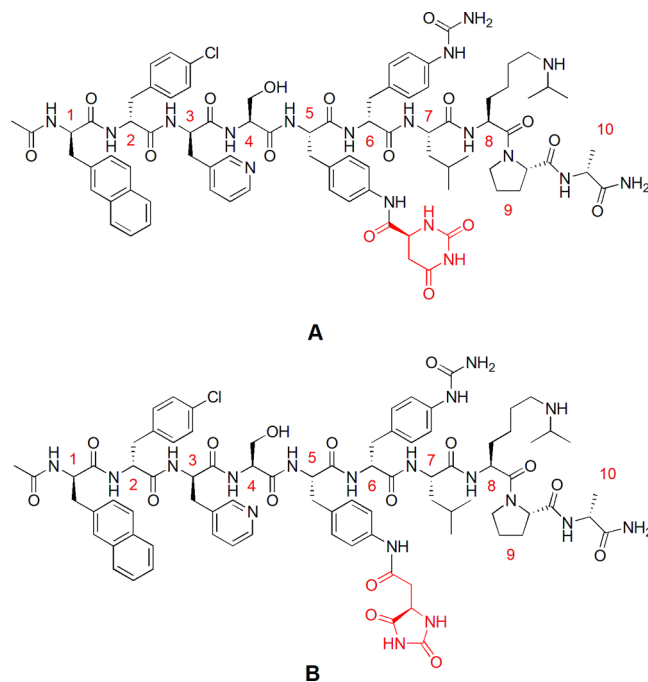
**KEYWORDS:** *Degarelix, dihydroorotic acid, Fmoc deprotection, peptide*

## INTRODUCTION

Degarelix is a synthetic peptide antagonist of the gonadotropin-releasing hormone, which was shown to be efficient for the treatment of advanced prostate cancer.<sup>1–3</sup> The peptide sequence of Degarelix contains several nonproteinogenic amino acids with particular properties, which determine the pharmacological action of this drug (Figure 1).<sup>4,5</sup>

In particular, the presence of the dihydroorotic moiety (Hor) in the side chain of one of the amino acids makes manufacturing of this peptide rather challenging. Indeed, the Hor group is highly prone to hydrolysis and rearrangement to the hydantoin (Hyd) structure in the presence of bases and water.<sup>6,7</sup> Due to a high similarity to the parent product, this impurity poses serious difficulties to the downstream process (purification), forcing the manufacturers to dramatically reduce the window of recovery during preparative high-performance liquid chromatography (HPLC). Similarly, the detection and quantification of this impurity in the purified Degarelix API (active pharmaceutical ingredient) result to be a complex task even with the most efficient equipment currently available such as ultra-performance liquid chromatography (UPLC). For all these reasons, the presence of the hydantoin isomer in the crude peptide drastically reduces the yield and productivity of the Degarelix manufacturing process and compromises the quality of the API launched in the market as well.

Because of the presence of several nonproteinogenic amino acids, Degarelix can be produced only by chemical approaches such as Boc or, more frequently, Fmoc solid-phase peptide synthesis (SPPS). The conventional methodologies of Fmoc-based SPPS include repetitive Fmoc deprotection cycles with the organic bases. In this case, piperidine is widely accepted as a reagent of the first choice. Unfortunately, these repetitive



**Figure 1.** Chemical structures of (A) Degarelix and (B) hydantoin isomer (Hor moiety and the corresponding hydantoin are evidenced in red).

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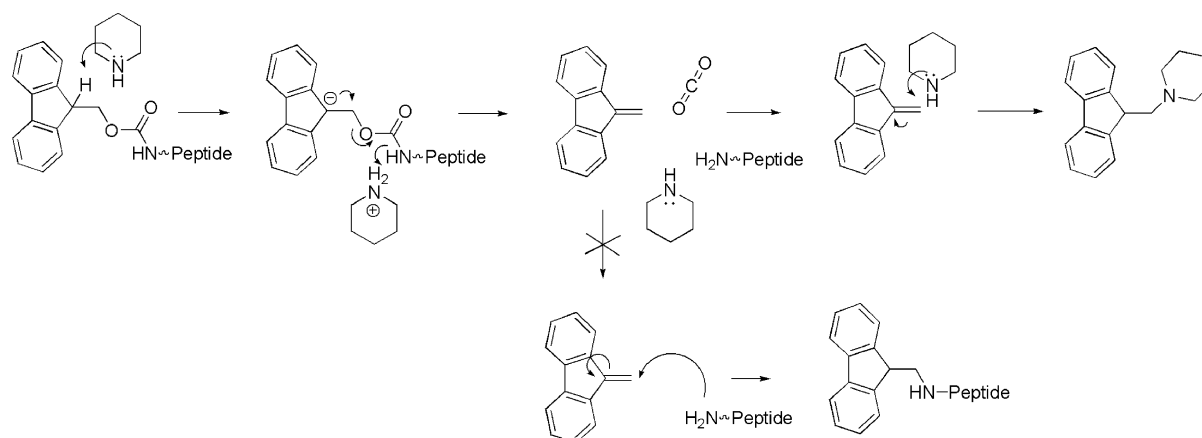


Figure 2. Scheme of Fmoc deprotection by piperidine.

Table 1. Trend of [Aph(Hyd)]<sup>5</sup>-Degarelix Formation in the Presence of Different Bases

Base	Chemical structure	pK <sub>a</sub>	Base in DMF, %	Monitoring time	Hydantoin, % (±0.05)	Hydantoin in the presence of 5% water, % (±0.05)
DBU		13.50	2	20 min	< 0.15	< 0.15
				1 h 40 min	0.69	1.38
				20 h	4.97	10.14
Pyrrolidine		11.27	20	20 min	< 0.15	< 0.15
				1 h 40 min	< 0.15	< 0.15
				20 h	< 0.15	< 0.15
Piperidine		11.22	20	20 min	< 0.15	0.22
				1 h 40 min	0.16	0.26
				20 h	0.18	0.67
<i>tert</i> -Butylamine		10.68	30	20 min	< 0.15	< 0.15
				1 h 40 min	< 0.15	< 0.15
				20 h	< 0.15	0.43
N-Methyl piperazine		9.14	5	20 min	< 0.15	< 0.15
				1 h 40 min	< 0.15	< 0.15
				20 h	< 0.15	< 0.15
Morpholine		8.36	50	20 min	< 0.15	< 0.15
				1 h 40 min	< 0.15	< 0.15
				20 h	< 0.15	< 0.15

basic treatments can induce the Hor-to-hydantoin rearrangement as it was demonstrated by Zhang et al.<sup>8</sup> using Degarelix API. The treatment of the peptide with standard Fmoc-deprotecting bases, such as 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) or piperidine with traces of water, resulted in the formation of up to 7% of hydantoin impurity. Thus, the use of high-quality anhydrous reagents is mandatory for Degarelix preparation. As reported by the same authors, piperidine shows a lower ability to induce the Hor–Hyd rearrangement than DBU. Unfortunately, epimerization is not completely avoided even with this base. Furthermore, piperidine is currently reported as a controlled substance in Table II of the United

Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances.<sup>9</sup> This aspect noticeably complicates the application of piperidine for industrial manufacturing of not only Degarelix but also the other peptides because of the rigid requirements applied for the purchase, storage, and disposal of this reagent.

To overcome all these problems, novel approaches are requested for manufacturing of Degarelix. In the course of our investigation on a Degarelix synthetic route, we previously proposed a novel method, where side-chain modification of the peptide is carried out on the solid phase from corresponding *p*-nitro derivatives, avoiding contact of Hor with the bases.<sup>10</sup> In

this study, we describe an alternative approach where Fmoc deprotection is carried out by means of the bases, which can minimize the Hor–Hyd rearrangement.

## RESULTS AND DISCUSSION

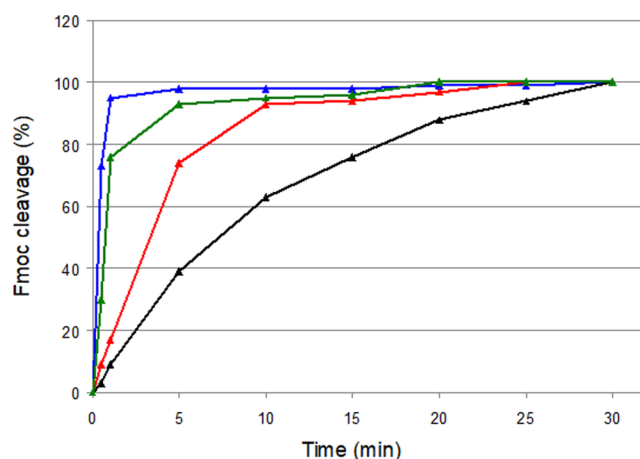
The Fmoc group, commonly used in SPPS, is cleaved under mild basic conditions by  $\beta$ -elimination.<sup>11,12</sup> The organic base of choice in Fmoc SPPS is a piperidine solution in dimethylformamide (DMF), or, alternatively, in “green” solvents.<sup>13</sup> Along with fast Fmoc deprotection, piperidine also functions as a scavenger for the formation of the side product dibenzofulvene, which is a highly reactive electrophile and can reattach to the liberated amine function (Figure 2).

However, several drawbacks of piperidine being used for the industrial production of peptides, as well as its possible induction of side reactions, triggered the search for suitable alternatives. In the case of Degarelix, the possibility of the Hor–Hyd rearrangement in the presence of bases significantly limits the choices of the deprotection mixture and therefore the applicability of Fmoc-based protections in the synthesis. In previous works, it was found that piperidine can be often substituted by other amines, for example, piperazine, morpholine, and diethylamine.<sup>14,15</sup> In order to select appropriately the organic base, which can be used for the Fmoc deprotection and at the same time does not induce Hor–Hyd rearrangement, we carried out a detailed screening of amines with different  $pK_a$  values (Table 1 and Figure S1).

The stability of Degarelix was tested in the presence of these amines as a function of time, ranging from 20 min (a standard, single Fmoc deprotection cycle) to 1 h 40 min (5 deprotection cycles to incorporate 4 amino acids after Aph(Hor) and to acetylate the peptide) and finally to 20 h. As expected, the treatment of Degarelix with half of the amines induced time-dependent hydantoin formation. In most of the cases, the addition of 5% water to the mixture resulted in an increased amount of the hydantoin impurity formed. The strongest base of the series, DBU, favored this rearrangement even in the absence of water as it was shown by Zhang et al.<sup>8</sup> Despite the low amount of hydantoin in the case of pyrrolidine, the degradation of the peptide, with appearance of other impurities, was observed. In particular, the mass spectrometry (MS) study showed the formation of an adduct of Degarelix with pyrrolidine, which may open the dihydroorotic cycle. Therefore, these bases were both excluded in further investigations. In the six-membered ring amine series, the influence of the  $pK_a$  of the base on the Hor moiety rearrangement was almost negligible (the amount of hydantoin ranged from 0.10 to 0.16%). Interestingly, no hydantoin impurity was found in the case of *tert*-butylamine probably due to its sterical hindrance and prevention of the deprotonation of the Hor moiety in the first step of the isomerization process.

The kinetics of Fmoc deprotection in the presence of the amines (with the exceptions of DBU and pyrrolidine) was studied using the Rink amide resin and model amino acids (Fmoc-Phe(*p*-NO<sub>2</sub>)-OH and Fmoc-Ser(*t*Bu)-OH) attached to the solid support (Figure 3 and Figures S2, S3).

As it was expected, the rate of Fmoc cleavage depended on the  $pK_a$  values of the bases. The strongest bases piperidine and *tert*-butylamine induced almost complete Fmoc cleavage in a few minutes. The same trend was observed for the Fmoc-protected Rink amide resin and *tert*-butyl ether side-chain-protected serine. On the contrary, the weaker bases morpholine and *N*-methylpiperazine performed Fmoc removal at a



**Figure 3.** Fmoc cleavage from Fmoc-Phe(*p*-NO<sub>2</sub>)-Rink amide resin (blue – piperidine, green – *tert*-butylamine, red – morpholine, black – *N*-methylpiperazine).

slower rate. Hence, although they do not induce the Hor–Hyd rearrangement, they may favor the formation of amino acid deletion products during the SPPS process of Degarelix.

Among the bases tested, *tert*-butylamine both afforded rapid cleavage of the Fmoc-protecting group and induced negligible Hor–Hyd rearrangement over a prolonged time period. For this reason, *tert*-butylamine was used in the Fmoc deprotection step of the full SPPS of Degarelix. The effect of *tert*-butylamine was investigated in the SPPS using both the preformed Fmoc-Aph(Hor)-OH amino acid and the *p*-nitro precursor, according to the strategy previously reported.<sup>10</sup> The results were compared with the full SPPS process using piperidine as a base for the Fmoc deprotection, focusing the attention on the Hor–Hyd rearrangement (Table 2 and Figures S4–S6).

**Table 2.** Results of SPPS Experiments of Degarelix Using *tert*-Butylamine or Piperidine as a Base for the Fmoc Deprotection Step

entry	strategy of SPPS Degarelix preparation	base used for Fmoc deprotection	HPLC purity(crude product), % ( $\pm 0.05$ )	[Aph(Hyd)] <sup>5</sup> -Degarelix, % ( $\pm 0.05$ )
1	full SPPS	<i>tert</i> -butylamine	87.50	<0.15
2	full SPPS <sup>8</sup>	piperidine	83.99	0.37
3	5-Phe( <i>p</i> -NO <sub>2</sub> )-Degarelix reduction and Hor coupling	<i>tert</i> -butylamine	88.63	<0.15
4	5-Phe( <i>p</i> -NO <sub>2</sub> )-Degarelix reduction and Hor coupling <sup>10</sup>	piperidine	87.04	<0.15

In both cases where *tert*-butylamine was used as a base (Table 2, entries 1 and 3), the crude peptide obtained showed high purity and yield without detectable [Aph(Hyd)]<sup>5</sup>-Degarelix impurity. This aspect is particularly remarkable for the full SPPS approach (entry 1) where the Hor function was inserted during the building up of the peptide sequence and not at the end. Furthermore, in contrast with piperidine, *tert*-butylamine has no concern from the regulatory and safety point of view; thus, it poses no restriction for the storage or use in the pharmaceutical industry. For these reasons, *tert*-butylamine can be considered as an excellent substitute to

piperidine for Fmoc deprotection in the solid-phase synthesis of the peptide. Both the *p*-nitro precursor reduction approach described in Part 1 of this study and the method where *tert*-butylamine is used give excellent results in terms of purity and yield when compared with the conventional stepwise synthesis. Though they give similar results, the latter approach seems to be more convenient from the industrial point of view since less unit operations are required to prepare the active pharmaceutical ingredient.

In conclusion, here, we evaluated a series of the organic bases that are able to remove the Fmoc-protecting group in order to substitute piperidine for the preparation of gonadotropin-releasing hormone antagonist Degarelix by solid-phase synthesis. Almost all the bases of this series induced either the rearrangement of the dihydroorotic moiety to hydantoin or the formation of other impurities. The only base capable to ensure both a fast Fmoc cleavage and negligible Hor–Hyd isomerization was *tert*-butylamine. Thus, this base can be considered as a suitable alternative to piperidine for SPPS manufacturing of Degarelix or other Hor-containing peptides using Aph(Hor) (both ready-to-use and assembled on resin) during the elongation of the amino acid sequence.

## EXPERIMENTAL PART

**Materials and Methods.** The following chemicals were purchased from Iris Biotech: *N,N*-dimethylformamide (DMF), dichloromethane (DCM), *N,N*-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), piperidine, and Fmoc-protected Rink amide resin; from Sigma-Aldrich: acetonitrile for MS (>99.9%), TFA for MS (>99.9%), methyl *tert*-butyl ether (MTBE), triisopropylsilane (TIS), and acetic anhydride; from Carbosynth: ethyl (hydroxyimino)cyanoacetate (Oxyma Pure) and *N,N'*-diisopropylcarbodiimide (DIC); from GL Biochem: Fmoc-Nal-OH, Fmoc-Cpa-OH, Fmoc-Pal-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-D-Aph(Cbm)-OH, Fmoc-Aph(Hor)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc, *i*Pr)-OH, Fmoc-Pro-OH, and Fmoc-D-Ala-OH; and from Merck: DBU, pyrrolidine, *tert*-butylamine, *N*-methyl piperazine, and morpholine.

UPLC analyses were performed on Agilent Technologies 1290 Infinity (stability experiments) and Agilent Technologies 1290 Infinity II instruments using an Acquity UPLC BEH C18 (1.7  $\mu$ m, 3  $\times$  150 mm) column. Analytical method 1: eluent A, TFA/H<sub>2</sub>O 0.1% v/v; eluent B: TFA/acetonitrile 0.1% v/v; detection at 224 nm; gradient mode: 0 min—25% of eluent B, 30 min—45% of eluent B. Analytical method 2: eluent A, TFA/H<sub>2</sub>O 0.1% v/v; eluent B: TFA/acetonitrile 0.1% v/v; detection at 224 nm; gradient mode: 0 min—25% of eluent B, 38 min—45% of eluent B, 47 min—60% of eluent B.

For mass spectrometry analysis, mass spectra were acquired in the LC–MS mode on an Agilent 6530 Accurate-Mass Q-ToF operating in the positive mode.

**Study of the Stability of Degarelix in the Presence of the Bases.** A sample of Degarelix API (130 mg/mL) was dissolved in a solution of the base in DMF. The aliquots were taken at different times and analyzed by UPLC.

**Study of the Kinetics of Fmoc Deprotection.** Fmoc-protected Rink amide resin (10 mg) was swelled in DMF for 15 min, and the selected amine was added to the suspension to obtain the required concentration and 1 mL of the total volume of the deprotection mixture. The reaction mixture was stirred, and samples of the solution (10  $\mu$ L) were taken after the desired time intervals. The samples were diluted by 990  $\mu$ L

of DMF in a 1 cm quartz cuvette. The absorbance was measured at 301 nm, and the loading was calculated by the formula  $L = (A_{301} \times V \times d) / (K \times w \times M)$  where  $L$  is the resin loading,  $A_{301}$  is the absorbance at 301 nm,  $V$  is the volume of the cleavage solution,  $K$  is the extinction coefficient (7800 mL mmol<sup>-1</sup> cm<sup>-1</sup>),  $w$  is the optical path length, and  $M$  is the weight (g) of the resin sample. In the same way, the experiments with Fmoc-Ser(*t*Bu)-Rink amide resin and Fmoc-Phe(*p*-NO<sub>2</sub>)-Rink amide resin were carried out.

**Peptide Synthesis. Synthesis of Degarelix with *tert*-Butylamine and Piperidine.** The synthesis was carried out using a CS-Bio 936 system and 250 g of Rink amide resin (loading of 0.65 mmol/g). The resin was swollen in 1.5 L of DMF for 30 min, Fmoc-deprotected with 30% *tert*-butylamine in DMF (three cycles for 5, 5, and 10 min; in the case of piperidine, all Fmoc deprotections were carried out with 20% piperidine in DMF, two cycles for 5 and 15 min), and washed four times with 1.5 L of DMF. The solid-phase synthesis was carried out using 3 equiv of protected amino acids that were preactivated for 3 min with 3 equiv of Oxyma Pure and 3 equiv of DIC and subsequently coupled in 1 h. The unreacted amino groups were capped with a 10% solution of acetic anhydride in DMF in 20 min up to D-Aph(Cbm), and at the end of the synthesis, the N-terminal amino group was acetylated using 3 equiv of acetic acid preactivated with 3 equiv of Oxyma Pure and 3 equiv of DIC for 3 min. The final acetylation was repeated twice. Peptide-resin was washed with DMF (2  $\times$  1.5 L) and DCM (2  $\times$  2 L), and the peptide was cleaved from the resin with 5 L of the mixture TFA/TIS/water (95:2.5:2.5 v/v) and precipitated in 10 L of MTBE. The product was filtered off and dried.

The identity of the peptide was confirmed by electrospray ionization MS:

Degarelix:  $[M+H]^+_{\text{calc}} = 1631.75$ ,  $[M+H]^+_{\text{exp}} = 1631.72$ . Yield (*t*-butylamine): 178 g (67%). Yield (piperidine): 172 g (65%).

**Synthesis of Degarelix with *tert*-Butylamine and 5-Phe(*p*-NO<sub>2</sub>)-Degarelix Reduction.** The synthesis was carried out as described previously with 500 g of Rink amide resin (loading of 0.65 mmol/g).<sup>10</sup> Instead of using piperidine for the Fmoc deprotection, 30% *tert*-butylamine in DMF was used (three cycles for 5, 5, and 10 min). Yield: 333 g (63%).

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://dx.doi.org/10.1021/acs.oprd.9b00540>

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## Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Steinberg, M. Degarelix: a Gonadotropin-Releasing Hormone Antagonist for the Management of Prostate Cancer. *Clin. Ther.* **2009**, *31*, 2312–2331.

(2) Doehn, C.; Sommerauer, M.; Jocham, D. Degarelix and its Therapeutic Potential in the Treatment of Prostate Cancer. *Clin. Interventions Aging* **2009**, *4*, 215–223.

(3) Clinton, T. N.; Woldu, S. L.; Raj, G. V. Degarelix Versus Luteinizing Hormone-Releasing Hormone Agonists for the Treatment of Prostate Cancer. *Expert Opin. Pharmacother.* **2017**, *18*, 825–832.

(4) Nestor, J., Jr. The Medicinal Chemistry of Peptides. *Curr. Med. Chem.* **2009**, *16*, 4399–4418.

(5) Zhou, N.; Gao, X.; Lv, Y.; Cheng, J.; Zhou, W.; Liu, K. Self-Assembled Nanostructures of Long-Acting GnRH Analogs Modified at Position 7. *J. Pept. Sci.* **2014**, *20*, 868–875.

(6) Kaneti, J.; Kirby, A. J.; Koedjikov, A. H.; Pojarlieff, I. G. Thorpe–Ingold effects in Cyclizations to Five-Membered and Six-Membered Rings Containing Planar Segments. The Rearrangement of N(1)-Alkyl-Substituted Dihydroorotic Acids to Hydantoinacetic Acids in Base. *Org. Biomol. Chem.* **2004**, *2*, 1098–1103.

(7) Koedjikov, A. H.; Blagoeva, I. B.; Pojarlieff, I. G.; Stankevici, E. J.  $\beta$ -Ureido Acids and Dihydrouracils. Part 15. Effect of Allylic Strain on Ring Opening of 1,6-Disubstituted Dihydrouracils. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1077–1081.

(8) Zhang, H.; Fomsgaarg, J.; Staerkaer, G. Method for the Manufacture of Degarelix. WO2010121835 A1, October 28, 2010.

(9) [https://www.incb.org/incb/en/precursors/technical\\_reports/2018.html](https://www.incb.org/incb/en/precursors/technical_reports/2018.html), 2018.

(10) ((a)) Guryanov, I.; Orlandin, A.; Biondi, B.; Formaggio, F.; Visentini, D. Process for the Manufacture of Degarelix and its Intermediates. WO2017103275 A1, June 22, 2017. (b) Guryanov, I.; Orlandin, A.; Viola, A.; Biondi, B.; Badocco, D.; Formaggio, F.; Ricci, A.; Cabri, W. Overcoming Chemical Challenges in the Solid-Phase Synthesis of High-Purity GnRH Antagonist Degarelix. Part 1. *Org. Process Res. Dev.* **2019**, *23*, 2746–2753.

(11) Fields, G. B. *Methods in Molecular Biology, Peptide Synthesis Protocols*; Pennington, M. W., Dunn, B. M., Eds.; Humana Press: Totowa, NJ, 1994; Vol. 35, pp. 17–27.

(12) Stigers, K. D.; Koutroulis, M. R.; Chung, D. M.; Nowick, J. S. Fmoc\*: a More Soluble Analogue of the 9-Fluorenylmethoxycarbonyl Protecting Group. *J. Org. Chem.* **2000**, *65*, 3858–3860.

(13) Kumar, A.; Thompson-Adewumi, A.; Nandhini, K. P.; Collins, J. M.; Albericio, F.; de la Torre, B. G. Troubleshooting When Using  $\gamma$ -Valerolactone (GVL) in Green Solid-Phase Peptide Synthesis. *Org. Process Res. Dev.* **2019**, *23*, 1096–1100.

(14) Wuts, P. G. M.; Greene, T. W. *Greene's Protective Groups in Organic Synthesis*; 4th Ed.; Wiley: Hoboken, NJ, 2007, pp. 712–713.

(15) Ralhan, K.; Krishna Kumar, V. G.; Gupta, S. Piperazine and DBU: a Safer Alternative for Rapid and Efficient Fmoc Deprotection in Solid Phase Peptide Synthesis. *RSC Adv.* **2015**, *5*, 104417.