2,2-Difluoro-1,3,2-oxazaborolidin-5-ones: novel approach for selective side-chain protection of serine and threonine

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2,2-Difluoro-1,3,2-oxazaborolidin-5-ones 1, which are synthesized from BF_3 and salts of amino acids, are highly effective, convenient and, moreover, inexpensive intermediates for the simultaneous protection of both α-amino and α-carboxy groups in α-amino acids. The new method streamlines the hitherto tedious procedures for side-chain protection of Ser and Thr. Ser(Bu^t), Thr(Bu^t), Ser(Bzl) and Thr(Bzl) are obtained by this procedure in high yields and in pure form using highly reactive reagents.

Introduction

Synthetic peptides are increasingly widely recognized as potential pharmaceutical agents and are of particular interest for the design, synthesis and evaluation of peptide libraries.[1] Therefore, there are growing demands for amino acid derivatives used as building-blocks in peptide synthesis which are not only chemically and stereochemically pure, but are also available at acceptable prices and prepared by convenient methods for practical large-scale synthesis. In our experience, the most troublesome preparations have involved the polyfunctional amino acids, especially in the side-chain protection of Asp, Glu, Ser, Thr and Tyr, *etc.*† Although these derivatives are commercially available, they are expensive and often impure.

Traditionally in peptide synthesis, the side-chain hydroxy functions in Ser and Thr have been protected as Bzl ethers based on N^{α}-Boc protection, and as Bu^t ethers in combination with an Fmoc group as the N^{α}-protecting group. In order to prepare Ser(Bu^t) and Thr(Bu^t), temporary protection of the α-amino and α-carboxy groups is necessary. In published procedures, the α-amino group has been blocked by a benzyloxycarbonyl group and the α-carboxy group by a *p*-nitrobenzyl ester[2] which is removable by hydrogenation after *tert*-butylation of the hydroxy groups. Moderate yields were achieved in both cases.

From the time when the Boc strategy was established, a Bzl group was selected as the protecting group for side-chain hydroxy functions in Ser and Thr. More recently, Fmoc-Ser(Bzl)-OH and Fmoc-Thr(Bzl)-OH have been popularly employed in convergent solid-phase peptide synthesis.[3] However, methods for preparation of Ser(Bzl) and Thr(Bzl) are even more complicated. Ser(Bzl)[4] and Thr(Bzl)[5] were first prepared by hydrolysis of the D,L-*N*-acetyl derivatives with an acylase, respectively. Although this method could give optically pure L-form products, it was not a practical method. Boc-Ser(Bzl)-OH was prepared by benzylation of Boc-Ser-OH under strong basic conditions.[6,7] The yield was moderate, but the procedure was not suitable for Thr protection; moreover, the Boc protecting groups had to be removed for preparation of Fmoc-Ser(Bzl)-OH. With regard to the synthesis of Thr(Bzl), the *O*-benzyl-L-threonine benzyl ester was prepared, followed by hydrolysis to give the desired compound.[8] Although the total yield was rather low (*ca.* 16%), the method was thought to be the only practical approach to Thr(Bzl).

Therefore, the major disadvantages associated with the earlier methods include tedious, multi-step protection and deprotection procedures, cumbersome reaction conditions and difficulty in scale-up. Furthermore, for Bzl side-chain protection, an effective etherification reagent is definitely necessary. For the above reasons, our studies have focused on α-amino acid intermediates with simultaneous protection of both α-functional groups. In this method the protected intermediates are prepared directly from free-form α-amino acids; free-form side-chain protected α-amino acids can be obtained after protection of the side-chain and deprotection of the intermediates. There are several methods which lead to simultaneous protection of both α-amino and α-carboxy groups in α-amino acids. For example, α-amino acids have been protected by Cu^{II} complexes,[9] by hexafluoroacetone,[10] or by boranes.[11,12] Although some amino acid derivatives have been synthesized through these intermediates, the applicability of these intermediates is limited due to their instability to acids or alcohols.

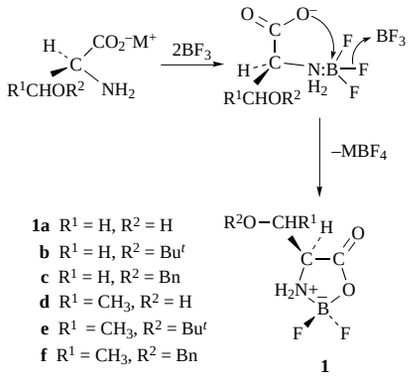
Being aware of the vast variety of boron complexes, which result from different substituents on the boron atom, we have prepared 4-substituted 2,2-difluoro-1,3,2-oxazaborolidin-5-ones **1**[13] (Scheme 1) from aspartic acid and glutamic acid, and reported the synthesis of Asp(OBu^t) and Glu(OBu^t) using **1** as intermediates which give simultaneous protection of both α-functional groups and are stable to Lewis acid catalysts. Here we describe an efficient and practical one-pot synthesis of the benzyl and *tert*-butyl-based side-chain protected serine and threonine derivatives which are useful in Boc and Fmoc chemistry, respectively.

Results and discussions

Formation, deprotection and properties of 1

The mono alkali-metal salt of the amino acid was treated with $BF_3{\cdot}Et_2O$ (>2 equiv.) in tetrahydrofuran (THF) to give **1** (see Scheme 1). The selection of salts is dependent on their ability to form an anhydrous compound. The lithium salts react with BF_3 in THF to form a clear solution and are thus preferred. However, for threonine, the sodium salt was selected because its lithium salt does not easily undergo dehydration. THF is the best solvent for the formation of **1**; other solvents such as ethylene glycol, dimethyl ether and DMF are also suitable depending on the solubility of compound **1** in them. The formation of compound **1** proceeds smoothly at room temperature, while at ele-

† All amino acids have the L-configuration except where indicated otherwise. Abbreviations are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature (*Biochem. J.*, 1967, **102**, 23; 1967, **104**, 17; 1972, **126**, 773): Boc = *tert*-butyloxycarbonyl, Bzl = benzyl, DMF = *N*,*N*-dimethylformamide, DMSO = dimethyl sulfoxide, Fm = fluoren-9-ylmethyl, Fmoc = fluoren-9-ylmethoxycarbonyl, OSu = *N*-hydroxysuccinimidyl and THF = tetrahydrofuran.



1a $R^1 = H, R^2 = H$
b $R^1 = H, R^2 = Bu^t$
c $R^1 = H, R^2 = Bn$
d $R^1 = CH_3, R^2 = H$
e $R^1 = CH_3, R^2 = Bu^t$
f $R^1 = CH_3, R^2 = Bn$

1

Scheme 1 Formation of 2,2-difluoro-1,3,2-oxazaborolidin-5-ones

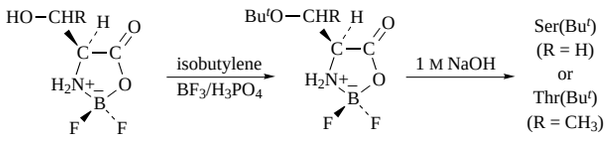
vated temperature the reaction can go to completion, normally at 40–50 °C, in 2–4 h.

The boron complex **1** is sensitive to moisture when the solvent is removed; therefore, the solvents used for the formation and the reaction of **1** should be anhydrous. On the other hand, compound **1** in solution is relatively stable. Reactions carried out under dry N_2 give best results. Pure intermediates were difficult to obtain because of the aforementioned reaction constraints; their properties remain, therefore, unknown. As mentioned above, deprotection of the compounds proceeds rapidly in water. In solution, the deprotection is accelerated in the presence of bases, while even in aqueous acid, compound **1** can be deprotected at room temperature within hours. These properties indicate that oxazaborolidinone **1** is a useful intermediate to effect protection irrespective of the sensitivity of the parent compound to acidic or basic conditions.

We have reported evidence for the formation of a 5-membered ring which incorporates both the α-functional groups of aspartic acid.[13] Here we report the ^{19}F NMR spectral results for compound **1a** which showed two multiplets, derived from the two fluorine atoms which became non-equivalent, being situated on either side of the molecular plane. The chemical shifts of the two different fluorine atoms in **1a** (from trifluoroacetic acid) were δ −74.92 and −75.41, respectively. Compounds **1a** and **1d** were directly used in the preparation of side-chain protected Ser and Thr derivatives without further purification.

Preparation of Ser(Bu^t) and Thr(Bu^t)

Compound **1a** or **1d** was allowed to react with isobutylene in dioxane with BF_3–H_3PO_4 as catalyst to give **1b** and **1e**, respectively. Hydrolysis with aqueous base then gave Ser(Bu^t) and Thr(Bu^t) (Scheme 2). In effecting *tert*-butylation, two important factors, the catalyst and solvent, should be considered. It was reported[14] that a combination of BF_3–H_3PO_4 was a mild catalyst and most effective in the preparation of *tert*-butyl ethers from Ser and Thr derivatives and isobutylene in CH_2Cl_2. With dioxane as the solvent, to achieve better results, more catalyst was needed compared with that for CH_2Cl_2 as solvent. At room temperature, the reaction was complete in 2–4 h. A prolonged reaction time was undesirable because of polymerization of the isobutylene. With THF as the solvent, even more catalyst was required and significant polymerization of isobutylene occurred.

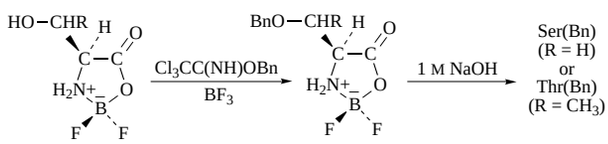


Scheme 2 Preparation of Ser(Bu^t) and Thr(Bu^t)

Amberlite XAD-2 or -4 non-ionic resin is useful in concentrating organic compounds in water.[15] We found they are also useful in the separation of amino acid derivatives from unsubstituted amino acids and inorganic salts in neutral aqueous solutions and, unlike ion-exchange resins, are able to separate some compounds sensitive to acids or bases. At pH 6, Ser(Bu^t) or Thr(Bu^t) was effectively adsorbed on the resin, while impurities arising from neutralization of BF_3, H_3PO_4 and oxazaborolidinones, were washed out from the resin by water. After eluting with aqueous alcohol, pure Ser(Bu^t) or Thr(Bu^t) can be isolated in high yields (Table 1). Fmoc-Ser(Bu^t)-OH was prepared from Fmoc-OSu and Ser(Bu^t),[16] which showed a similar optical rotation value to that reported.[17]

Preparation of Ser(Bzl) and Thr(Bzl)

Compound **1a** or **1d** was allowed to react with benzyl trichloroacetimidate[18] in dioxane with BF_3 as the catalyst, to give **1c** or **1f**, respectively. The desired compounds can be obtained after deprotection of the intermediates (Scheme 3). In order to avoid strong basic conditions, benzyl trichloroacetimidate was used to effect the etherification. As previously reported, benzyl trichloroacetimidate was commonly used in non-polar solvents, such as cyclohexane or carbon tetrachloride, in order to avoid its rearrangement to the corresponding *N*-benzylamide. In this study, we found that dioxane was also a suitable solvent for the reaction. Within 0.5–2 h, the yield was >90% in both cases with no more than 1.2 equiv. of benzyl trichloroacetimidate; this indicated that rearrangement was insignificant. We also found that Amberlite XAD-2 or -4 was a more effective adsorbent for Ser(Bzl) and Thr(Bzl) than for Bu^t-protected derivatives. Therefore, Ser(Bzl) and Thr(Bzl) can likewise also be purified by Amberlite XAD-2 or -4 (Table 1). Because Ser(Bzl) and Thr(Bzl) are not very soluble in cold water, they can be separated simply by filtration. Fmoc-Thr(Bzl)-OH was prepared according to the literature.[16] Finally, the optical purities of Ser(Bzl) and Thr(Bzl) were also examined on an HPLC column with a chiral crown ether as the stationary phase; the results showed that no detectable racemization occurred in the preparations.



Scheme 3 Preparation of Ser(Bzl) and Thr(Bzl)

In summary, the present study demonstrates that 2,2-difluoro-1,3,2-oxazaborolidin-5-ones are highly effective, convenient and, moreover, inexpensive intermediates for simultaneous protection of both α-amino and α-carboxy groups in α-amino acids. The new method has streamlined the hitherto tedious procedures for side-chain protection of Ser and Thr, and will pave the way for further applications of this technique to access many variants of side-chain modified amino acids, especially *O*-glycosylated amino acids.[22]

Experimental

General information

Mps were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.) and the $[\alpha]_D^{25}$ values are given in 10^{-1} deg cm^2 g^{-1}. ^1H (400, 500 MHz) and ^{13}C (100, 125 MHz) NMR spectra were recorded either on a Bruker DPX 400 or ARX 500 spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard (δ values). *J* Values are given in Hz. ^{13}C Signal assignments for each signal were established by measurements of 2D spectra; multiplicities are indicated by p (primary), s (secondary), t (tertiary) or q (quaternary). Analytical TLC was carried out on Merck pre-

Table 1 Properties of the Ser and Thr derivatives

	Ser(Bu^t)	Thr(Bu^t)	Ser(Bzl)	Thr(Bzl)
Mp/°C				
Found	225–226 (decomp.)	234–236 (decomp.)	221–222 (decomp.)	203–204 (decomp.)
Reported	220[20]	244–247[20]	219–221[21]	197[5]
$[\alpha]_D^{25}$	(*c* 1.0 in H_2O)	(*c* 1.0 in MeOH)	(*c* 1.0 in 3N HCl)	(*c* 1.0 in HOAc)
Found	−17.0	−44.6	+6.0	−30.8
Reported	−16.5 ± 1[20]	−43 ± 2[20]	+5.86[4]	−30.4[8]
Elemental analysis (%) (Calc.)	C 51.13 (51.01)	C 54.15 (54.14)	C 61.31 (61.53)	C 62.87 (63.14)
	H 9.58 (9.42)	H 9.64 (9.79)	H 6.60 (6.71)	H 7.16 (7.22)
	N 8.34 (8.50)	N 7.85 (7.89)	N 7.12 (7.18)	N 6.75 (6.70)
Empirical formula	$C_7H_{15}NO_3{\cdot}\frac{1}{5}H_2O$	$C_8H_{17}NO_3{\cdot}\frac{1}{8}H_2O$	$C_{10}H_{13}NO_3$	$C_{11}H_{15}NO_3$
Yield (%)	85.6–92.4	73.6–86.7	94.8	90.8

coated silica gel plates (Kieselgel G 60 F_{254}) employing the solvent system, BuOH–AcOH–H_2O (4:1:1, v/v). Non-aqueous reactions were carried out under a nitrogen atmosphere. THF and dioxane were dried and redistilled from calcium hydride. MeOH was distilled from magnesium. Boron trifluoride–diethyl ether was dried (CaH_2) and distilled at reduced pressure. Isobutylene was dried ($CaSO_4$) at −20 °C overnight. Salts of amino acids were lyophilized with FZ-12 Freeze Dry System (Labconco.) and then warmed to 80 °C for 2 h at 3–24 × 10^{-3} mmHg. Amberlite® XAD-4 adsorbent (Merck) was washed with water (2 vol. of the resin), MeOH (2 vol.) and water (2 vol.) before use. Anhydrous phosphoric acid (crystals 98+%) was purchased from the Aldrich Chemical Co., Inc. Amino acids, D,L-Ser(Bzl) and D,L-Thr(Bzl) were obtained from Watanabe Chemical Industries, Ltd. Other laboratory reagents were used directly without further purification. Benzyl trichloroacetimidate was prepared according to the literature[18] with modification: benzyl trichloroacetimidate was purified by distillation *in vacuo* (0.4 mmHg) to give a colourless liquid and was used in the preparation of benzyl derivatives immediately after its purification.

2,2-Difluoro-4-hydroxymethyl-1,3,2-oxazaborolidin-5-one 1a and 2,2-difluoro-4-(2-hydroxyethyl)-1,3,2-oxazaborolidin-5-one 1d

To the salt of the amino acid (mono lithium salt for Ser, mono sodium salt for Thr) (10 mmol) suspended in THF (15 cm^3), was added $BF_3{\cdot}Et_2O$ (6.0 cm^3). The mixture was stirred at room temperature for 6 h and then at 40–45 °C for an additional 2 h, to give **1a** or **1d** in quantitative yield.

H-Ser(Bu^t)-OH

A solution of **1a** (obtained from 10 mmol of Ser) in THF, which was prepared as above in a 50 cm^3 Pyrex Erlenmeyer flask, was concentrated to dryness with the aid of a water pump at room temperature. After removal of the solvent, the evaporation was continued for an additional 0.5 h, after which dioxane (30 cm^3) was added to the residue. The solution was stirred for 15 min after which H_3PO_4 (98+%; 0.4 cm^3) was added, and stirring was continued for an additional 15 min. The flask containing **1a** was then cooled to −20 °C and isobutylene (20 cm^3) was added at −20 °C. The flask was securely stoppered to ensure no leakage of isobutylene. The resultant solution was stirred at room temperature for 2–2.5 h. After discharge of isobutylene, the solution was poured into a stirred solution of 1 mol dm^{-3} aqueous NaOH (80 cm^3) at 0 °C and stirring was continued for 0.5 h. The mixture was then evaporated *in vacuo*, the residue was dissolved in water (30 cm^3) and the solution extracted with diethyl ether (3 × 15 cm^3). The aqueous solution was adjusted to pH 6.0 with 1 mol dm^{-3} HCl, after which the solution was chromatographed on Amberlite XAD-4 resin (∅ 2.5 × 34 cm). The resin was washed with water and then 80% EtOH. The latter eluent containing Ser(Bu^t) was collected and evaporated to give a white solid (1.38–1.49 g).

H-Thr(Bu^t)-OH

The title compound was prepared from **1d** (prepared from 10 mmol of Thr) by following a similar procedure for preparation of Ser(Bu^t) except for the amount of isobutylene (25 cm^3) and the reaction time for *tert*-butylation (2.5–3.0 h). After isolation on Amberlite XAD-4 resin, Thr(Bu^t) was obtained as a white solid (1.29–1.52 g).

Fmoc-Ser(Bu^t)-OH

The title compound was prepared from Ser(Bu^t), which was obtained as above, and Fmoc-OSu[16]; mp 126–128 °C; $[\alpha]_D^{25}$ +25.5 (*c* 1.0, EtOAc) [lit.,[17] mp 126–129 °C $[\alpha]_D^{25}$ +25.4 (*c* 1.0, EtOAc)] (Found: C, 68.8; H, 6.53; N, 3.75. Calc. for $C_{22}H_{25}NO_5$: C, 68.9; H, 6.57; N, 3.65%); δ_H(500 MHz, $CDCl_3$) 1.18 (9 H, s, Bu^t), 3.62 (1 H, dd, *J* 8.8, 4.0, OCHH_aCH), 3.90 (1 H, dd, *J* 8.8, 2.7, OCHH_bCH), 4.24 [1 H, t, *J* 7.0, 9-H, fluorenylmethyl (Fm)], 4.37 (1 H, dd, *J* 10.7, 7.2, CH_aH, Fm), 4.42 (1 H, dd, *J* 10.7, 7.4, CH_bH, Fm), 4.51 (1 H, dt, *J* 8.3, 4.1, NCHCO), 5.71 (1 H, d, *J* 8.3, CONH), 7.30 (2 H, t, *J* 7.4, 2- and 7-H, Fm), 7.39 (2 H, t, *J* 7.4, 3- and 6-H, Fm), 7.61 (2 H, t, *J* 6.9, 1- and 8-H, Fm) and 7.75 (2 H, d, *J* 7.5, 4- and 5-H, Fm); δ_C(125 MHz, $CDCl_3$) 27.3 (p, 3 × C, Bu^t), 47.1 (t, 9-C, Fm), 54.4 (t, 2-C, Ser), 61.8 (s, 3-C, Ser), 67.3 (s, CH_2, Fm), 74.1 [q, O-*C*$(CH_3)_3$], 120.0 (t, 2 × C, 4- and 5-C, Fm), 125.1, 125.2 (t, each 1 × C, 1- and 8-C, Fm), 127.1 (t, 2 × C, 2- and 7-C, Fm), 127.5 (t, 2 × C, 3- and 6-C, Fm), 141.3 (q, 2 × C, 4a- and 4b-C, Fm), 143.7, 143.9 (q, each 1 × C, 8a- and 9a-C, Fm), 156.3 (q, CONH) and 175.1 (q, CO_2H).

H-Ser(Bzl)-OH

A THF solution of **1a** (10 mmol) was evaporated *in vacuo* at room temperature to remove the solvent. After most of the THF had been removed, the residue was kept *in vacuo* (1.0 mmHg) for an additional 0.5 h. Dioxane (30 cm^3) was added to the residue and the solution was stirred for 10 min. To this solution, benzyl trichloroacetimidate (2.15 cm^3, 11.5 mmol) was added over 10 min. After being stirred for 2 h, the mixture was treated with anhydrous methanol (5 cm^3), stirred for 10 min and then heated with 1 mol dm^{-3} aqueous NaOH (30 cm^3); it was then stirred for an additional 30 min. The residue obtained when the mixture was evaporated *in vacuo* was dissolved in water (200 cm^3) and the resulting solution was washed with diethyl ether (3 × 15 cm^3). The aqueous phase was adjusted to pH 6.0 and passed through a column containing Amberlite XAD-4 resin (∅ 2.5 × 20 cm). The column was washed with water and then 50% EtOH. The latter eluent containing the desired compound was collected and evaporated *in vacuo* to give a white solid (1.85 g); δ_H[500 MHz, $(CD_3)_2SO$] 3.47 (1 H, dd, *J* 7.6, 3.4, NCHCO), 3.67 (1 H, dd, *J* 10.4, 7.7, OCH_aHCH), 3.79 (1 H, dd, *J* 10.4, 3.4, OCH_bHCH), 4.51 (2 H, s, CH_2Ph) and 7.28–7.41 (5 H, m, phenyl); δ_C[125 MHz, $(CD_3)_2SO$] 54.0 (t, 2-C, Ser), 69.0 (s, CH_2Ph), 72.0 (s, 3-C, Ser), 127.4 (t, 4-C, phenyl), 127.6, 128.1 (t, each 2 × C, 2-, 3-, 5- and, 6-C, phenyl), 137.9 (q, 1-C, phenyl) and 167.6 (q, CO_2H).

H-Thr(Bzl)-OH

The title compound was prepared from **1d** (10 mmol) by following the procedure as described in the preparation of Ser(Bzl) above, except for the amount of benzyl trichloroacetimidate (2.3 cm^3, 12.0 mmol); 1.9 g product was obtained; δ_H[500 MHz, $(CD_3)_2SO$] 1.24 (3 H, d, *J* 6.5, CH_3), 3.15 (1 H, d, *J* 4.6, $NCHCO_2$), 4.03 (1 H, dq, *J* 4.6, 6.4, $OCHCH_3$), 4.47, 4.53 (each 1 H, each d, *J* 11.9, OCH_2Ph), 7.25–7.29 (1 H, m, 4-H, phenyl) and 7.31–7.37 (4 H, m, 2-, 3-, 5- and 6-H, phenyl); δ_C[125 MHz, $(CD_3)_2SO$] 17.6 (p, CH_3), 58.6 (t, 2-C, Thr), 70.3 (s, CH_2ph), 73.4 (t, 3-C, Thr), 127.2 (t, 4-C, phenyl), 127.6, 128.0 (t, each 2 × C, 2-, 3-, 5- and 6-C, phenyl), 138.4 (q, 1-C, phenyl) and 168.3 (q, CO_2H).

Fmoc-Thr(Bzl)-OH

The title compound was prepared from Thr(Bzl), obtained as above, and Fmoc-OSu according to the literature,[16] mp 115–116 °C; $[\alpha]_D^{25}$ +11.5 (*c* 1.0, MeOH) [lit.,[19] mp 114–117 °C, $[\alpha]_D^{25}$ +11.2 (*c* 1.0, MeOH)] (Found: C, 72.2; H, 5.93; N, 3.23. Calc. for $C_{26}H_{25}NO_5$: C, 72.4; H, 5.84; N, 3.25%); δ_H(400 MHz, $CDCl_3$) 1.25 (3 H, d, *J* 6.3, CH_3), 4.20–4.24 (2 H, 9-H, Fm and $OCHCH_3$), 4.36–4.47 (4 H, $NCHCO_2$, CH_2-Fm and CH_aH-phenyl), 4.58 (1 H, d, *J* 11.6, CH_bH-phenyl), 5.63 (1 H, d, *J* 9.4, COHN), 7.22–7.31 (7 H, phenyl and 2- and 7-H, Fm), 7.38 (2 H, t, *J* 6.8, 3- and 6-H, Fm), 7.60 (2 H, t, *J* 6.8, 1- and 8-H, Fm), 7.74 (2 H, d, *J* 7.5, 4- and 5-H, Fm), 9.56 (1 H, br s, CO_2H); δ_C(100 MHz, $CDCl_3$) 16.2 (p, CH_3), 47.1 (t, 9-C, Fm), 58.5 (t, 2-C, Thr), 67.4 (s, CH_2-Fm), 71.2 (s, CH_2Ph), 74.2 (t, 3-C, Thr), 120.0 (t, 2 × C, 4- and 5-C, Fm), 125.1, 125.2 (each t, 1- and 8-C, Fm), 127.1 (t, 2 × C, 2- and 7-C, Fm), 127.7 (t, 2 × C, 3- and 6-C, Fm), 127.8, 128.0, 128.4 (t, 5 × C, 2-, 3-, 4-, 5- and 6-C, phenyl), 137.5 (q, 1-C, phenyl), 141.3 (q, 2 × C, 4a- and 4b-C, Fm), 143.7, 143.9 (each q, 8a- and 9a-C, Fm), 156.8 (q, CONH) and 175.6 (q, CO_2H).

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