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Synthesis of β-methyl-β-alanine-L-proline-XAA tripeptides by $Yb(OTf)_3$ catalysed Michael addition of amines to *N*-crotonyl-L-proline-XAA: a versatile route to cyclic β-methyl-β-alanine-derived tripeptides via ring closing metathesis

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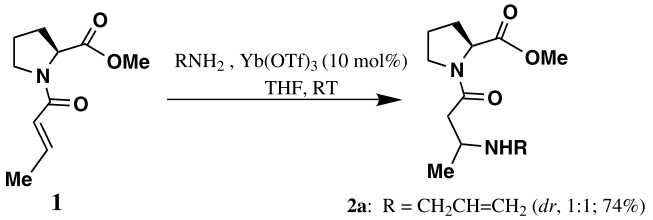
Abstract—*N*-Crotonyl-L-proline derived peptides can be transformed to the corresponding β-methyl-β-alanine-L-proline peptides by $Yb(OTf)_3$ catalysed Michael addition of aliphatic amines to the crotonyl residue. The Michael adducts derived from addition of allylamine are versatile precursors to the corresponding cyclic peptides obtainable via ring closing metathesis.

Peptides derived from β-amino acids are resistant to proteolytic degradation and therefore of considerable importance in medicinal chemistry.[1] A peptide bond derived from α- and β-amino acids also leads to molecules with interesting conformational properties.[2] In an ongoing project in our laboratory,[3] we required peptides consisting of a β-amino acid and L-proline to probe their proteolytic stabilities towards aspartyl proteases.[4] In order to develop a synthetic methodology for a dipeptide, derived from β-methyl-β-alanine-L-proline we used *N*-crotonoyl-L-proline as a precursor for their synthesis. This communication describes our preliminary results on $Yb(OTf)_3$ catalysed synthesis of β-methyl-β-alanine-L-proline containing dipeptides.

$Yb(OTf)_3$ is known to catalyse the Michael addition of amines to crotonate esters.[5] In order to probe the synthetic scope of this reaction, we explored the Michael addition of different alkyl amines to *N*-crotonoyl-L-proline[6] methyl ester under $Yb(OTf)_3$ catalysis. Accordingly, allylamine, benzylamine and 3-bromobenzylamine were subjected to Michael addition with *N*-crotonoyl-L-proline methyl ester **1** in the presence of catalytic (10 mol%) $Yb(OTf)_3$ leading to the formation of β-methyl-β-alanine-L-proline containing amides **2** (Scheme 1).[7] As is evident from the results in Scheme 1, amide **1** underwent smooth Michael addition in high yields (70–75%) to the corresponding amide **2**, however, the reaction was not diastereoselective as mixtures of both isomers were obtained in equal proportion. In order to improve the diastereoselectivity during the Michael addition, we carried out additions using the corresponding *N*-crotonoyl-L-proline dipeptides **3** (Scheme 2), reasoning that these may preorganise via a γ-turn leading to a less flexible structure which will result in a facial bias for Michael addition. An ^1H NMR study of peptides **3** indicated the presence of an intramolecular hydrogen bond suggesting that **3a** and **3b** are indeed preorganised via a γ-turn. **3a** and **3b** were subjected to $Yb(OTf)_3$ catalysed Michael addition using allylamine leading, after *N*-protection, to *N*-butoxycarbonyl (Boc) peptides **4a** and **4b** in good yields (Scheme 2).



2a: R = $CH_2CH{=}CH_2$ (*dr*, 1:1; 74%)
2b: R = $C_6H_5CH_2$ (*dr*, 1:1; 76%)
2c: R = 3-$BrC_6H_4CH_2$ (*dr*, 1:1; 69%)

Scheme 1. $Yb(OTf)_3$-catalysed Michael addition of amines.

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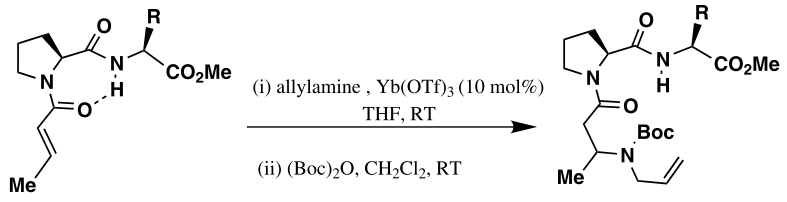


3a: R = benzyl
3b: R = isobutyl

4a; (*dr*, 60: 40; 67%)
4b; (*dr*, 55:45; 70%)

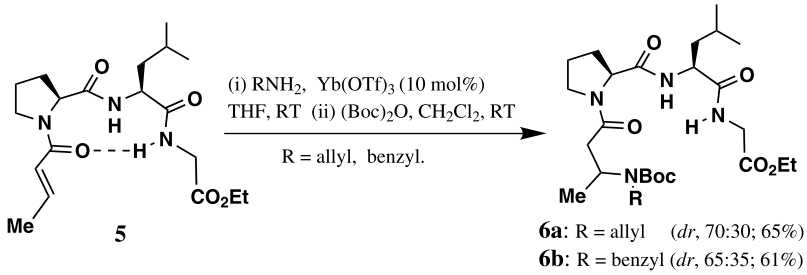
Scheme 2. $Yb(OTf)_3$-catalysed Michael addition of allylamine.

However, a careful analysis of the reaction mixture by ^1H NMR indicated it to be a mixture (60:40) of diastereomers meaning that the γ-turn present in **3** is not able to exert any meaningful control during Michael addition of amines. In order to further improve the diastereoselectivity, we synthesised peptides capable of existing as β-turns. It is known that a β-turn preorganises peptides into conformationally constrained, less flexible conformations. Such preorganised β-turns in a peptide may provide a better facial selectivity in the crotonoyl group leading to high diastereoselectivity during $Yb(OTf)_3$ catalysed Michael addition of alkyl amines.

Thus the corresponding dipeptide *N*-crotonoyl-L-proline-leucine–glycine ethyl ester **5** was synthesised according to standard amide coupling procedures and the existence of γ- and β-turns[8] in this peptide was probed by deuterium exchange studies[9] using CD_3OD in $CDCl_3$ (Scheme 3). There was no deuterium exchange for the bonded amide proton even after 5 hours, clearly indicating the presence of strong intramolecular hydrogen bonding in **5**. When **5** was treated with allylamine or benzylamine under $Yb(OTf)_3$ catalysed conditions, followed by protection, *N*-Boc dipeptides **6a** and **6b** were obtained in good yields (Scheme 3). However the diastereoselectivity in this reaction had improved only marginally (70:30) indicating only a limited role for the preorganised structure, in influencing the transition state of the Michael addition. The peptides **4** and **6** could be separated into pure diastereomers and in spite of the moderate selectivity during the Michael addition, this methodology provided an efficient route for the synthesis of both diastereomers of β-methyl-β-alanine-L-proline-XAA tripeptides. With allylamine at the β-amino acid terminus in these peptides we realised that the introduction of another double bond at the other end of the peptide would lead to a suitable precursor for ring closing metathesis (RCM).

Thus we wanted to constrain these peptides containing a β-methyl-β-alanine-L-proline bond, by cyclisation. The cyclic peptides derived from such residues are attractive compounds as pharmaceutical probes for proteases. Also a comparative study of the acyclic as well as the corresponding cyclic peptide may provide vital information regarding the 'bioactive conformation' of such structures.

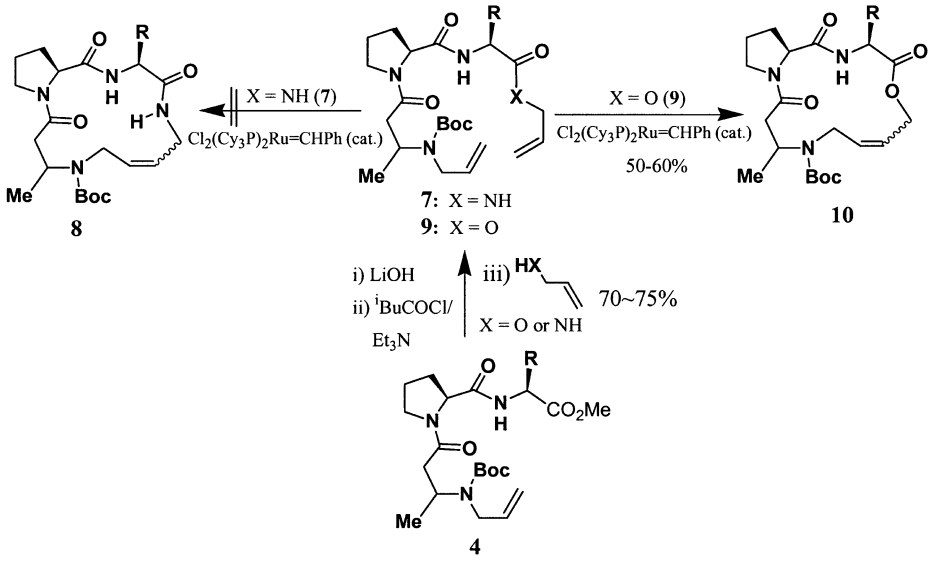
In view of the importance of the cyclic peptides, we undertook a systematic study on the cyclisation of these peptides by RCM.[10] Thus the dipeptides **4** were converted to the corresponding allylamides **7** via routine synthetic manipulation (Scheme 4) and the diastereomers were separated by column chromatography. In view of the probability that the allylamides **7** exist with a β-turn, we first explored their cyclisation using Grubbs' ruthenium catalyst. Surprisingly no cyclised peptide **8** was observed with either of the diastereomers (**7a** and **7b**) under RCM conditions. Molecular dynamics simulation studies showed that allylamides **7** are organised via a conformation that does not permit the two terminal double bonds to come into close proximity for cyclisation (Fig. 1). Accordingly, we turned our attention to the corresponding allyl esters **9** with the premise that these may exist in a favourable conformation (Fig. 1). Thus both the diastereomers of **9** (**9a** and **9b**) were separated by column chromatography and subjected to RCM studies. Our assumption was vindicated as both **9a** and **9b** underwent smooth RCM with Grubbs' catalyst leading to the corresponding cyclic peptides **10a** and **10b**, respectively. Similarly the diastereomers **9c** and **9d** were separated and subjected to RCM to afford the corresponding cyclic peptides **10c** and **10d**, respectively. It is noteworthy that all the diastereomers i.e. **9a–d** underwent RCM with equal ease and efficiency (Table 1).



5

6a: R = allyl (*dr*, 70:30; 65%)
6b: R = benzyl (*dr*, 65:35; 61%)

Scheme 3. $Yb(OTf)_3$-catalysed Michael addition of amines to peptide **5**.



Scheme 4. RCM of tripeptides **7** and **9**.

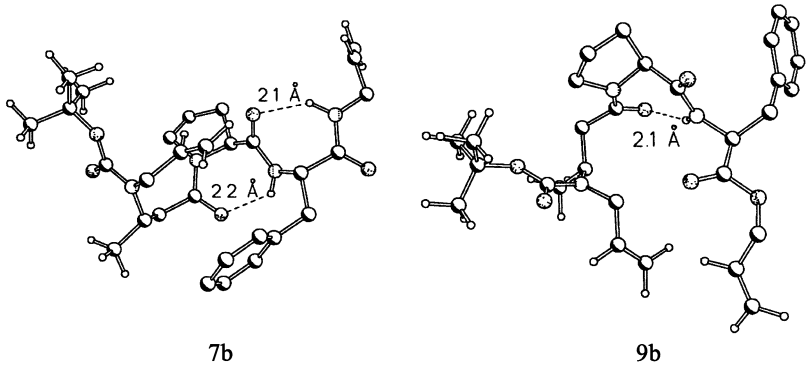
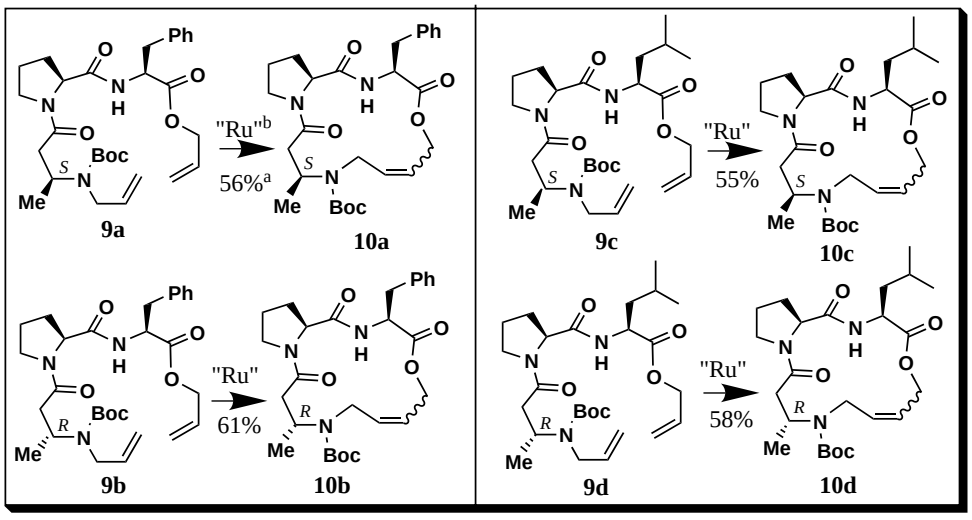
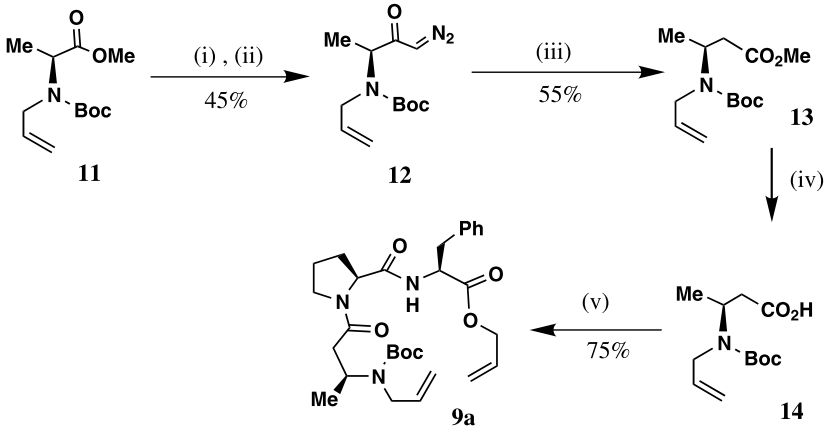


Figure 1. Energy minimised structures show preference for the allyl esters **9** as compared to allyl amides **7** in RCM cyclisation.

Table 1. Ru-alkylidine catalysed RCM on the diastereomers of tripeptides **9**



a) yield of the isolated cyclic peptides b) "Ru"= $Cl_2(Cy_3P)_2Ru=CHPh$



Scheme 5. Assignment of the configuration of the β-amino acid in **9a**. *Reagents and conditions*: (i) LiOH, THF:H_2O, rt; (ii) isobutyl chloroformate, Et_3N, CH_2N_2 (excess), CH_2Cl_2 at 0°C; (iii) Ag(OAc), Et_3N, MeOH, rt; (iv) LiOH, THF:H_2O; (v) EDC, HOBT, *O*-allyl-L-phenylalanine-L-proline, Et_3N, CH_2Cl_2, 0°C to rt.

The cyclic peptides were obtained as a mixture of *E* and *Z* isomers in which the former isomer was the major product (*E*:*Z* = 3:1).

In order to ascertain the absolute stereochemistry of the chiral centre of the β-amino acid, we have carried out the chemical correlation study described in Scheme 5. We synthesised *N*-allyl-*N*-Boc-L-alanine **11** starting from L-alanine and made the corresponding diazoketone **12** by mixed anhydride protocols using isobutyl chloroformate and diazomethane in dichloromethane. Arndt–Eistert homologation on **12** using AgOAc gave β-amino ester **13**, which was hydrolysed to afford the corresponding β-amino acid **14**. Coupling the pure acid **14** with L-proline-L-phenylalanine allyl ester yielded the pure diastereomer. Optical rotation and HPLC studies indicated that the compound obtained by homologation ($[\alpha]_D$ −31.3, *c* 1.15 in $CHCl_3$) (Scheme 5) is identical with the polar diastereomer **9a** ($[\alpha]_D$ −37.8, *c* 0.65 in $CHCl_3$) obtained by $Yb(OTf)_3$ catalysed Michael addition of allylamine with **3a**. Therefore the polar diastereomers of **4a** and **9** were assigned the '*S*' absolute stereochemistry at the chiral centre in the β-amino acid residue. Similarly the '*S*' assignment was made for the other polar diastereomer **9c** and the corresponding cyclic peptides (i.e. **10a** and **10c**). The less polar diastereomers were accordingly assigned the '*R*' configuration at the β-amino residue in **9b**, **9d**, **10b** and **10d**.

In conclusion, this paper describes a novel synthetic route for β-methyl-β-alanine-L-proline derived peptides using $Yb(OTf)_3$ catalysed Michael addition of amines to *N*-crotonoyl-L-proline or *N*-crotonoyl-L-proline peptides, respectively. The facile addition of allylamine leads to the dipeptide containing β-methyl-β-alanine-L-proline residue which are useful precursors for cyclic peptides obtainable by RCM.

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6. **General procedure for *N*-crotonoyl-L-proline-XAA peptide synthesis:** To a stirred solution of *N*-crotonoyl-L-proline acid in THF at 0°C was added triethylamine (1 equiv.), followed by isobutyl chloroformate (1 equiv.) and the mixture vigorously stirred for 5 min and then to this was added XA′A′-allyl amide/ester followed by triethylamine (1 equiv.). The reaction mixture was stirred for 5 h at rt and after that the solvent was removed, the residue taken up

in ethyl acetate, washed with sodium bicarbonate and saturated citric acid solution and finally with brine. Drying over sodium sulfate and concentration in vacuo yielded the crude product which was subjected to column chromatography (silica gel, EtOAc:hexane) to afford the desired peptide in good yield.

7. **General procedures for Michael addition reaction of amines to methyl *N*-crotonoyl L-prolinate followed by Boc protection:** To a stirred solution of methyl *N*-crotonoyl L-prolinate **1** (0.4 g, 1 equiv., 2.0 mmol) and benzylamine (0.22 mL, 0.21 g, 1 equiv., 2.0 mmol) or 3-bromobenzylamine (generated prior to the reaction by neutralising 3-bromobenzylamine hydrochloride (0.5 g, 1.1 equiv., 2.2 mmol), with an excess of $NaHCO_3$) or allylamine (0.2 mL, 0.15 g, 1.3 equiv., 2.6 mmol) in THF (1.0 mL/mmol) was added $Yb(OTf)_3$ (0.125 g, 0.1 equiv., 0.2 mmol) at room temperature and the reaction mixture was stirred for 12 h. After that the reaction mixture was diluted with hexane and filtered through a celite pad, the filtrate was concentrated under vacuum, and passed through a filtration column (silica gel, 2% MeOH in ethyl acetate). This mixture was subjected to di-*tert*-butyl dicarbonate (Boc_2O), (0.45 g, 2.06 mmol), catalytic *N*,*N*-dimethylaminopyridine in dichloromethane and after aqueous work-up dried and evaporated to give a crude residue. Chromatographic purification (silica gel, EtOAc:hexane) of the crude material gave the desired amines **2** as a diastereomeric mixture.
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10. (a) Nguyen, S. T.; Johnson, L. K.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3974; (b) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed.* **1997**, *36*, 2036–2056 and References cited therein; (c) Furstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012–3043 and References cited therein; (d) Phillips, A. J.; Abell, A. D. *Aldrichim. Acta* **1999**, *32*, 75–90; (e) Flink, B. E.; Kym, P. R.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 4334–4344. ***General procedure for RCM*:** To a stirred solution of Grubbs' ruthenium catalyst (10 mol%) in dry dichloromethane (60 mL) under a nitrogen atmosphere was added the di-allylated peptide **9** (1 mmol) dissolved in dry dichloromethane (40 mL) slowly over a period of 30 min and then refluxed. After 12 h another portion of catalyst (10 mol%) was added to the reaction mixture and refluxing continued for another 12 h. After this the reaction was exposed to air and directly subjected to column chromatography (silica gel, EtOAc:hexane) to afford the corresponding cyclic product **10** as a mixture of *E* and *Z* isomers in 50–60% yield. ***Spectral data for some selected compounds*:** **4a:** Oil; Yield: 67%; FTIR ($CHCl_3$): 3313, 2976, 1746, 1686, 1528 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.25 (d, 3H, $J=5.9$ Hz), 1.45 (s, 9H), 1.85–2.01 (m, 3H), 2.25–2.67 (m, 2H), 2.95–3.38 (m, 5H), 3.71 (s, 3H), 3.75–3.91 (m, 2H), 4.1–4.20 (m, 2H), 4.53–4.84 (m, 2H), 5.07–5.19 (m, 2H), 5.72–5.78 (m, 1H), 7.14–7.26 (m, 5H), 7.40 (bs, 1H); CI MS *m*/*z* (*iso*-butane): 502 (*M*+1), 470, 444, 402, 370, 277, 170. **9a:** Oil; yield: 76%; $[\alpha]_D$ −37.8, (*c* 0.65, $CHCl_3$); FTIR ($CHCl_3$): 3311, 2976, 1744, 1687, 1527, 1366 cm^{-1}; ^{1}HNMR (200 MHz, $CDCl_3$): δ 1.23 (d, 3H, $J=7.2$ Hz), 1.47 (s, 9H), 1.77–2.04 (m, 4H), 2.31–2.41 (m, 2H), 3.00 (dd, 1H, $J=7.4$, 13.5 Hz), 3.19 (dd, 1H, $J=5.9$, 13.6 Hz), 3.42 (bs, 2H), 3.68–3.87 (m, 2H), 4.15–4.45 (m, 1H), 4.50–4.76 (m, 3H), 4.81 (dd, 1H, $J=6.9$, 13.7 Hz), 5.07–5.34 (m, 4H), 5.76–5.92 (m, 2H), 7.13–7.27 (m, 5H), 7.44 (bs, 1H); CI MS *m*/*z* (*iso*-butane): 528 (*M*+1), 514, 470, 428, 370, 342, 303, 170. **10a:** solid; yield: 56%; mp: 157–158°C; $[\alpha]_D$ −110.0 (*c* 0.15, MeOH); FTIR ($CHCl_3$): 3314, 3010, 2962, 2928, 1736, 1683, 1516 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.44 (d, 3H, $J=7.2$ Hz), 1.51 (s, 9H), 1.70–1.91 (m, 4H), 2.30–2.36 (m, 2H), 2.61–2.76 (m, 1H), 3.2 (dd, 1H, $J=5.2$, 14.2 Hz), 3.42–3.50 (m, 2H), 3.64–3.94 (m, 2H), 4.12–4.35 (m, 1H), 4.45 (dd, 1H, $J=6.7$, 11.2 Hz), 4.54–4.72 (m, 2H), 5.01 (dd, 1H, $J=7.0$, 13.5 Hz), 5.66–5.70 (m, 1H), 5.78–5.86 (m, 1H), 6.0 (brd, 1H, $J=13.3$ Hz), 7.1 (d, 1H, $J=7.1$ Hz), 7.24–7.30 (m, 4H); CI MS *m*/*z* (*iso*-butane): 500, 484, 426, 400 (100%), 388; **10b:** Solid; yield: 61%; mp: 183–184°C; $[\alpha]_D$ −82.6 (*c* 0.5,$CHCl_3$); FTIR ($CHCl_3$): 3314, 3009, 2962, 2929, 1737, 1683, 1516 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, δ ppm): 1.44 (d, 3H, $J=8.2$), 1.53 (s, 9H), 1.69–1.90 (m, 4H), 2.30–2.39 (m, 2H), 2.93 (m, 1H), 3.25 (dd, 1H, $J=5.2$, 14.2), 3.40–3.50 (m, 2H), 3.64–3.94 (m, 2H), 4.14–4.37 (m, 1H), 4.45 (dd, 1H, $J=6.6$, 11.2), 4.54–4.7 (m, 2H), 5.0 (dd, 1H, $J=7.0$, 13.7), 5.65–5.71 (m, 1H), 5.78–5.86 (m, 1H), 6.01 (brd, 1H, $J=13.3$), 7.12 (d, 1H, $J=7.1$), 7.24–7.30 (m, 4H); CI MS *m*/*z* (*iso*-butane): 500 $(M+H)^+$, 484, 444, 426, 400 (100%), 388, 149; **10c:** Oil; yield: 55%; $[\alpha]_D$ −58.9 (*c* 0.1, $CHCl_3$); FTIR ($CHCl_3$): 3307, 3010, 2965, 2874, 1743, 1685, 1534 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, δ ppm): 0.90 (bs, 6H), 1.27 (d, 3H, $J=6.8$), 1.44 (s, 9H), 1.57–2.5 (m, 9H), 3.5–4.13 (m, 5H), 4.5–4.62 (m, 4H), 5.06–5.6 (m, 4H), 5.73–5.91 (m, 1H), 6.0–6.2 (m, 1H); CI MS *m*/*z* (*iso*-butane): 466, 450, 410, 394, 366, 354, 344.