Bioorganic & Medicinal Chemistry Letters 21 (2011) 4768–4772

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological properties of macrolactam analogs of the natural product macrolide (−)-A26771B

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ARTICLE INFO

Article history:
Received 28 April 2011
Revised 14 June 2011
Accepted 15 June 2011
Available online 23 June 2011

Keywords:
Natural products
(−)-**A26771B**
Antibacterial
Macrolactams
Metathesis

ABSTRACT

Promising synthetic derivatives of macrolactone natural product (−)-**A26771B** have been designed and synthesized both from semisynthesis and total synthesis. Further optimization led to the first synthesis of macrolactam analogs of (−)-**A26771B** with improved antibacterial activity and metabolic stability.

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Natural product **(−)-A26771B** produced by *Penicillium turbatum* has been identified as an attractive target in the development of new antibiotics.[1] **(−)-A26771B** exhibits moderate in vitro activity against Gram-positive bacteria, mycoplasma and fungi. Despite its attractive biological properties, **(−)-A26771B** suffers from poor pharmacokinetic properties which translates into a lack of in vivo activity after subcutaneous administration against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* infections in mice. Promising results obtained from a screening campaign with synthetic derivatives of **(−)-A26771B** encouraged us to explore further the structure–activity relationship (SAR) in order to identify candidates with improved biological profile and pharmacokinetic properties. We wish to report herein our results on the synthesis and evaluation of the biological activity of new analogs which led to the first total synthesis of macrolactam analogs of **(−)-A26771B**.

(−)-**A26771B** is a structurally unique 16 member ring macrolactone (Fig. 1) possessing a highly oxidized γ-oxo-δ-hydroxy-α,β-unsaturated carboxyl system found in many other natural macrocycles such as cladospolides, cytochalasins, colletodiols and macrosphelides.[2] Although the total synthesis of **(−)-A26771B** has been well covered,[3] limited SAR was available from literature data. We anticipated that the sensitivity of the 4-oxygenated 2-enoic carboxyl functionality could be a risk from a chemical and metabolic stability and potentially toxicological perspective (general Michael acceptor). Our first goal aimed to measure the impact of replacing this reactive functionality. We explored at first a semisynthetic strategy to access the first analogs. For this purpose, multigram quantities of pure **1** could be obtained from *P. turbatum* fermentation.

The first analogs were prepared from the *t*-butyl succinate analog **2** (Scheme 1), easier to handle on a chemistry perspective and which turned out to be at least as active as **(−)-A26771B** against a panel of bacteria (Table 1). Hydrogenation of **2** provided the saturated analog **3** with good yield. 1,4-Addition of various amines to the α,β-unsaturated carbonyl system was regioselective and proceed smoothly to provide compounds **4** and **5a**. Compound **5b** was obtained by acylation in the presence of acetic anhydride. No control was observed on the newly created stereogenic center during 1,4-addition. Reaction of **2** with TMS-diazomethane followed by treatment with tetrabutyl ammonium fluoride (TBAF) provided the heterocyclic pyrazole derivatives **6** over two steps al-

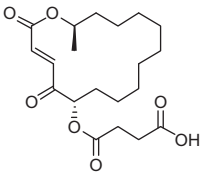

Figure 1. (−)-**A26771B** (**1**).

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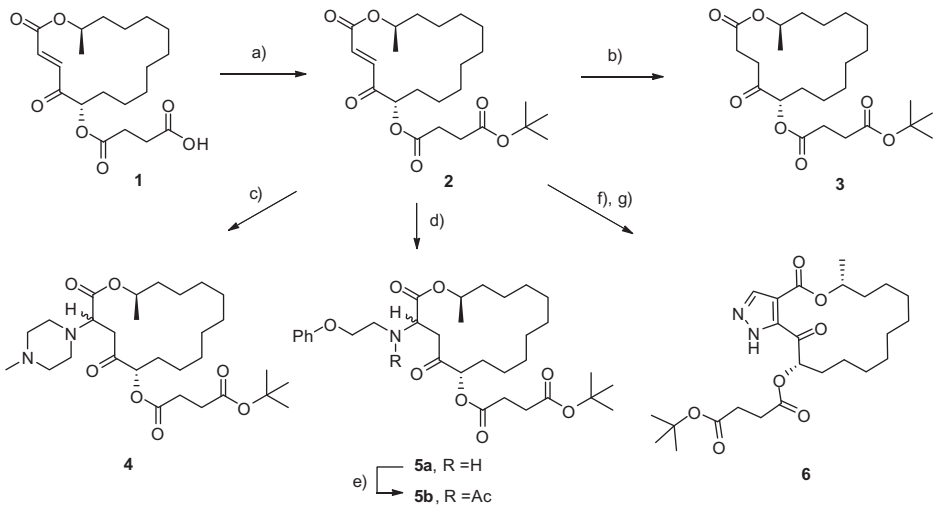
doi:10.1016/j.bmcl.2011.06.073



Scheme 1. Analog synthesis via semisynthesis. Reagents and conditions: (a) *t*-BuOCNHCCl$_3$, CH_2Cl_2, 40 °C; 88%; (b) H_2, Pd/C, EtOAc, rt; 94%; (c) *N*-methyl piperazine, CH_2Cl_2, rt; 62%; (d) $C_6H_5OC_2H_4NH_2$, CH_2Cl_2, rt; used as crude; (e) Ac_2O, Et_3N, CH_2Cl_2, 0 °C to rt; 46% over two steps; (f) TMS-diazomethane, $Pd(OAc)_2$, CH_2Cl_2/Et_2O, 0 °C; used as crude; (g) TBAF, THF, rt; 24% over two steps.

Table 1
MIC values (μg/mL)[a]

Bacterial strains	1	2	4	5b	6	7	8	9	10	13	14	15	16a	16b	17	18	19	28a	28b
Escherichia coli ATCC 25922	>32	>32	>32	>32	>64	>32	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Enterococcus faecalis ATCC29212	16	2	4	>32	>64	2	2	4	32	>64	>64	>64	64	>64	>64	4	4	2	2
Enterococcus faecium 1	4	nd	4	>32	>64	8	2	2	16	>64	>64	>64	32	>64	>64	4	4	0.5	0.5
Haemophilus influenzae ATCC 31517	>32	>32	>32	>32	>64	16	32	32	>64	>64	>64	>64	>64	>64	>64	32	32	2	2
Haemophilus influenzae LS2 efflux knock-out	16	8	nd	>32	8	1	4	4	16	64	>64	>64	>64	>64	2	2	2	0.25	0.25
Pseudomonas aeruginosa ATCC 27853	>32	>32	>32	>32	>64	>32	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Staphylococcus aureus ATCC13709	2	2	2	>32	>64	1	1	0.5	32	>64	>64	>64	32	>64	1	1	1	1	0.5
Staphylococcus aureus Sa2 MRSA[c]	2	0.5	2	>32	>64	2	0.5	0.5	32	>64	>64	>64	>64	>64	2	2	1	1	0.5
Staphylococcus aureus ATCC25923	4	8	4	>32	>64	2	1	1	32	>64	>64	>64	64	>64	1	1	1	1	1
Staphylococcus aureus ATCC25923 +10%SH[b]	>32	>32	>32	>32	>64	32	16	16	>64	>64	>64	>64	>64	>64	16	32	32	8	8
Staphylococcus aureus Oxford	2	2	2	>32	>64	1	1	1	32	>64	>64	>64	64	>64	1	1	1	1	0.5
Streptococcus pneumoniae ATCC49619	16	2	4	>32	>64	2	2	2	16	>64	>64	>64	64	>64	2	4	4	2	2
Streptococcus pneumoniae Pen9 IV381-221	8	1	2	>32	32	2	2	4	8	>64	>64	>64	64	>64	1	2	4	8	8

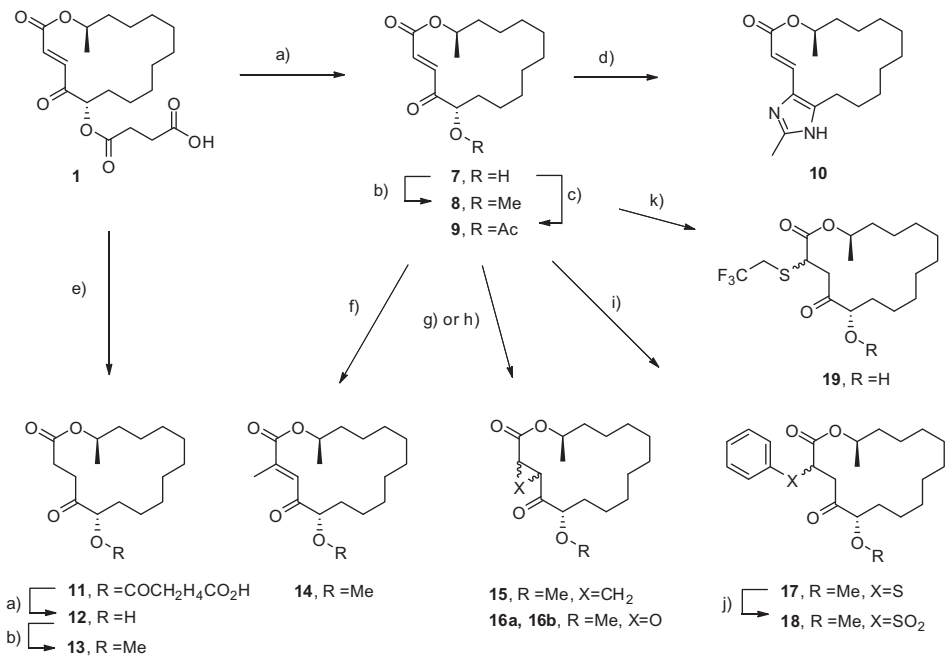
[a] Minimum inhibitory concentration. Determined according to the CLSI method by broth microdilution.
[b] 10% of human serum were added to the media.
[c] Clinical strain.

beit in moderate yield (24%). We then focused our program on functionalizing the hydroxyl group with more varied chemical functionalities as shown in Scheme 2. Saponification of **1** under smooth conditions provided the poorly stable hydroxyl derivative **7** which could not be purified by flash chromatography. This crude product was therefore used without further purification in the following steps.

Methylation of **7** with methyl iodide provided the methoxy derivative **8** while acylation with acetic anhydride provided the acetate **9**. These two compounds were more stable than the free hydroxyl derivative and were used as the starting point to access more radical modifications of the natural product framework. The imidazole derivative **10** was obtained from **9** by treatment with ammonium acetate in refluxing acetic acid. Furthermore, the saturated analogs of **(−)-A26771B** were prepared as the succinate **11**, free hydroxyl **12** and methoxy derivatives **13** after a sequence of hydrogenation, saponification and methylation. Modification of the 4-oxygenated 2-enoic carboxyl functionality was further investigated to address both the SAR and to improve the poor metabolic stability of **(−)-A26771B**. The sterically hindered methyl substituted derivative **14** was obtained from a two step procedure implying a 1,4-addition of a methyl cuprate to the double bond and quenching the resulting enolate with phenylselenium bromide. Oxidation of the crude selenide intermediate with hydrogen peroxide provided compound **14**, albeit in low yield (4% over two steps).

Cyclopropane **15** was obtained in low yield as a mixture of two diastereomers using the conditions described by Taylor.[4] A slightly better yield was obtained upon epoxidation with *t*-butyl hydroperoxide to provide compound **16** as a mixture of *trans*-diastereomers which were separated by flash chromatography. The extreme sensitivity of the 4-oxygenated 2-enoic carboxyl functionality is illustrated by the very fast addition of phenyl thiol to the ene-system in dichloromethane at rt. Thioether **17** was further oxidized to the sulfone in the presence of *m*-CPBA while the trifluoroethyl thioether **19** was prepared as the free hydroxyl derivative in low yield due principally to difficulty of isolation.

Although the synthesis of analogs of **(−)-A26771B** from semisynthesis allowed us to prepare a large number of compounds and to better understand the SAR around the unique 16 member ring macrolactone, the quest for metabolically more stable compounds encouraged us to explore the synthesis of lactam deriva-

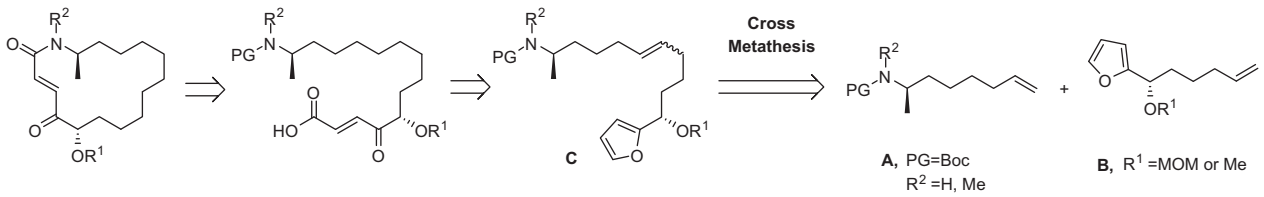


Scheme 2. Analog synthesis via semisynthesis. Reagents and conditions: (a) LiOH, THF/H_2O, 0 °C; used as crude; (b) MeI, Ag_2O, CH_2Cl_2, rt; 35% from **1**; (c) Ac_2O, pyridine, CH_2Cl_2, 0 °C to rt; 46% from **1;** (d) NH_4OAc, AcOH, rt to 80 °C; 32% from **9**; (e) H_2, Pd/C, EtOAc, rt; 89%; then conditions (a) 71% for **11** and (b); 46% for **12**; (f) MeLi, CuI, Et_2O, −40 °C then PhSeBr, THF, −40 °C to rt; mixture of compounds isolated in 11% yield and then submitted to H_2O_2, pyridine, CH_2Cl_2, 0 °C to rt; 38%; (g) Me_3SOI, DBU, CH_3CN, 0 °C; 14% from **8**; (h) TBHP, Triton B, Tol. 0 °C to rt; 30% from **8**; (i) PhSH, CH_2Cl_2, rt; 56%; (j) *m*-CPBA, CH_2Cl_2, rt; 85%; (k) CF_3CH_2SH, CH_2Cl_2, rt; 18% from **1**.

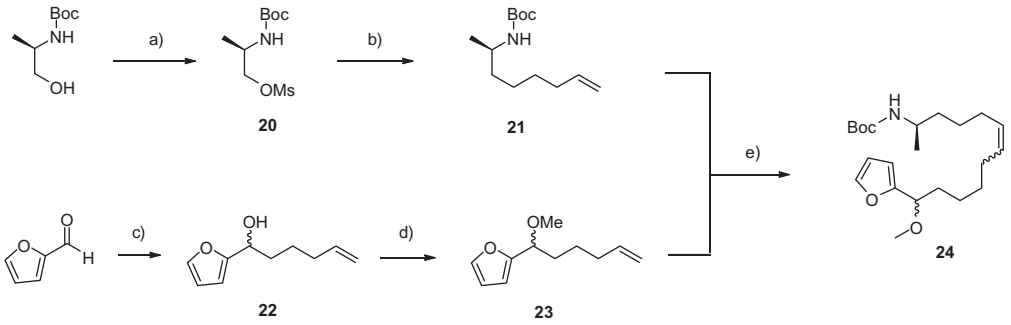
tives. Preparation of lactam derivatives from a parent macrolactone has been previously exemplified for an epothilone in the literature.[5] However, a short procedure could not be envisioned in the case of **(−)-A26771B**. Furthermore, we anticipated some chemistry limitations due to the high reactivity of the 4-oxygenated 2-enoic functionality. A total synthesis of macrolactam analogs was therefore envisaged. Our retrosynthetic analysis is highlighted in Scheme 3. A macrolactamization comparable to the cyclization step performed by Chang[3k] was chosen as the final step. We then decided to take advantage of the elegant methodology developed by Kobayashi[3h,j] to introduce the 4-oxygenated 2-enoic acid system from a 2-substituted furan. We thought that this key furan intermediate **C** could readily be accessible via a cross-metathesis[6a] disconnection not previously reported for the total synthesis of **(−)-A26771B**. This very concise approach would allow us to reach the desired 16 member ring macrolactam in seven sequential steps. The first intermediate **21** was readily prepared in enantiomerically pure form in two steps from the commercially available Boc-protected (*R*)-alaninol (Scheme 4).

Racemic compound **23** was obtained in two steps and quantitative yield as described by Chang et al.[3k] As anticipated, cross-metathesis of intermediates **21** and **23** afforded the desired compound **24** in very good yield (83%). 15% Grubbs I catalyst[6b,c] in dichloromethane at room temperature turned out to be the ideal conditions. A fivefold excess of furan derived olefin **23** was used to avoid homodimerization of olefin **21**.[7] This excess was recovered during purification. Compound **24** was obtained as a mixture of four stereoisomers which were hydrogenated to provide **25** as an inseparable mixture of two isomers. Opening of the furan ring under Kobayashi's conditions[3h,j] followed by sodium chlorite oxidation of the formed aldehyde provided the key intermediate acid **27** as a mixture of two diastereomers. The macrolactamization was performed under dilute conditions (10^{-2} M) after Boc-deprotection, to give the expected lactam **28** in 25% non-optimized overall yield (Scheme 5). The two diastereomers were separated at this stage by flash chromatography.[8,9]

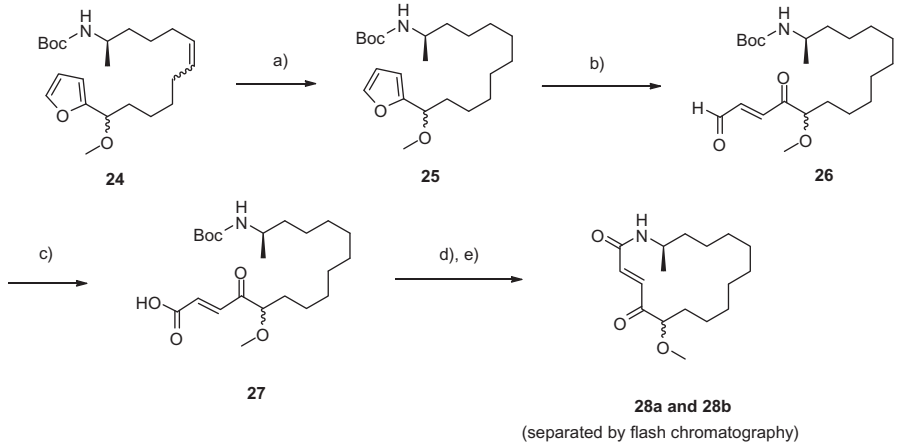
The antibiotic activity of **(−)-A26771B** analogs was then assessed against a panel of various bacterial strains (Table 1). As mentioned previously, the *t*-butyl succinate **2** as well as the acetate derivative **9** appeared to have a profile similar to the natural product with a slightly more pronounced effect on the *S. aureus* MRSA strain (MIC = 0.5 μg/mL). As similar results were obtained with the free hydroxyl **7**, we concluded that the succinate moiety in the natural product had no obvious effect on the activity. This hypothesis was confirmed by the synthesis of the methoxy derivative **8** which turned out to be slightly more active than the natural product **1**. Furthermore all our attempts to further stabilize the molecule by modification of the sensitive 4-oxygenated 2-enoic carboxyl functionality led to a virtually complete loss of the antibacterial activity (compounds **6**, **10**, **13–16**). It is worth noting that



Scheme 3. Retrosynthetic analysis for the total synthesis of (−)-**A26771B**.



Scheme 4. Synthesis of intermediate **24** via Grubbs I catalyzed cross-metathesis. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, −10 °C; 98%; (b) CuI, C_5H_9MgBr, THF, −15 °C; 48%; (c) C_5H_9MgBr, THF, 0 °C; quant.; (d) NaH, MeI, THF, 0 °C to rt; quant.; (e) Grubbs I (15 mmol %), CH_2Cl_2, rt; 83%.



Scheme 5. Synthesis of compound **28** via macrolactamization. Reagents and conditions: (a) H_2, Pd/C, AcOEt, rt; 97%; (b) NBS, $NaHCO_3$, acetone/H_2O, −15 °C, 30 min then pyridine, rt; 66%; (c) $NaClO_2$, isopentene, *t*-BuOH; quant.; (d) TFA, CH_2Cl_2, rt; used without purification; (e) HATU, HOBt, DIPEA, DMAP, CH_2Cl_2, rt; 25%.

although the acylated amino derivative **5b** was also inactive, the activity was rescued by the introduction of a basic nitrogen β to the ketone as exemplified by the *N*-methyl piperazine analog **4** (this result was confirmed with other *sec*- and *tert*-amine derivatives: data not shown). In addition, phenylthioether **17**, phenyl sulfone **18** and trifluoroethyl thioether **19** had an antibacterial activity similar to the methoxy analog **8**. As the four compounds **4, 17**, **18, 19** share a strong sensitivity toward beta-elimination, we believe they could act as prodrugs by their capacity to regenerate in situ the 4-oxygenated 2-enoic carboxyl functionality which seems to be critical to maintain the antibacterial activity. Macrolactams **28a** and **28b** were then evaluated against the same panel of bacterial strains. Both compounds were active against a broad spectrum of bacteria and **28b** was revealed as the most active compound identified within these series. Worth noting is the activity of **28a** and **28b** on *Haemophilus influenzae* ATCC 31517 and *H. influenzae* LS2 Efflux Knock-out while natural product **(−)-A26771B** was inactive. Furthermore, unlike analogs derived from natural macrolactones, the antibacterial activity of these two compounds was successfully maintained in the presence of serum in the screening assay (*S. aureus* ATCC25923). This important result opened encouraging perspectives of 16 member ring macrolactams to access biologically active compounds with improved pharmacological properties. Unfortunately, progress obtained in vitro did not translate into an orally active candidate. No activity was observed after oral administration of **28a** and **28b** at a dose of 50 mg/kg in a lung infection model.

To conclude, we achieved the synthesis of various analogs of natural product **(−)-A26771B**. The SAR has been established and emphasizes the role of the sensitive 4-oxygenated 2-enoic carboxyl functionality in the antibiotic activity. The first synthesis of macrolactam analogs was achieved via a new cross-metathesis approach. These compounds revealed a more pronounced antibacterial activity as compared to the natural product **(−)-A26771B**.

Acknowledgments

We would like to thank Drs. Phil Dudfield, Alfred Greiner, Jean-Yves Ortholand and Fritz Hansske for valuable discussions. We would like to thank Kathleen Sonck and Carole Delachaume for their contribution to the microbiological study. We are also thankful to BioFocus, Allschwil, for providing substantial amounts of pure natural product (−)-**A26771B** to complete the semisynthetic program. And finally, we would like to thank Dr. Richard Jarvest and David Holmes (GSK) for constructive discussions and for providing us with the *H. influenzae* LS2 strain.

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7. To a solution of Grubbs I catalyst (179 mg, 0.22 mmol, 0.15 mol %) in CH_2Cl_2 (170 mL, degazed by N_2 for 10 min) was added via canula a solution of **21** (330 mg, 1.46 mmol, 1.0 equiv) and **23** (1.30 g, 7.21 mmol, 5.0 equiv) in CH_2Cl_2 (100 mL, degazed by N_2 for 10 min) at rt over 50 min. The reaction mixture was stirred at rt for 24 h and more Grubbs I catalyst (70 mg, 0.085 mmol) was added. After 4 h, the reaction mixture was concentrated under vacuum. Expected product (463 mg, 83%, mixture of 4 stereoisomers) was isolated by flash chromatography (pentane/Et_2O: 9/1) as a brownish oil.
8. Stereochemistry of each diastereomer **28a** and **28b** could not be assigned by NMR analysis.
9. A solution of **27** (50 mg, 0.12 mmol) in CH_2Cl_2 (1 mL) at room temperature was treated with TFA (0.1 mL, 1.3 mmol, 12.0 equiv) and stirred for 70 min. The reaction mixture was then concentrated under vacuum. The crude residue was dissolved in CH_2Cl_2 (12 mL) and HATU (55 mg, 0.144 mmol, 1.2 equiv), HOBt (10 mg, 0.072 mmol, 0.6 equiv), a catalytic amount of DMAP and DIPEA (126 μL, 0.72 mmol, 6.0 equiv) were added successively. The reaction mixture was stirred at room temperature for 2 h then water was added. The 2 layers were separated. The aqueous phase was extracted with CH_2Cl_2. The combined organics were washed with a saturated aqueous solution of $NaHCO_3$, with a saturated aqueous solution of NH_4Cl and with brine then dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was purified by column chromatography on silica gel (pentane/EtOAc: 6/4–5/5) to give **28a** (4 mg, 11%) and **28b** (5 mg, 14%) as colourless oils.
Compound **28a**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.48 (d, 1H, J = 15.5 Hz), 6.78 (d, 1H, J = 15.5 Hz), 5.47 (br d, 1H, J = 9.2 Hz), 4.24 (m, 1H), 3.76 (dd, 1H, J = 7.6 and 4.6 Hz), 3.36 (s, 3H), 1.85–1.60 (m, 2H), 1.38–1.02 (br s, 16H), 1.20 (d, 3H, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 202.6 (s), 164.1 (s), 134.6 (d), 132.5 (d), 86.5 (d), 57.9 (q), 45.6 (d), 35.8 (t), 30.9 (t), 28.3 (t), 28.1 (t), 27.9 (t), 27.5 (t), 27.1 (t), 23.8 (t), 22.2 (t), 21.1 (q); MS (ES^+) 296 (M+H), 318 (M+Na), 591 (2M+H), 613 (2M+Na); (ES^-) 294 (M−H), 340 (M+$HCOO^-$).
Compound **28b**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.15 (d, 1H, J = 15.5 Hz), 6.92 (d, 1H, J = 15.5 Hz), 5.65 (br d, 1H, J = 9.0 Hz), 4.21 (m, 1H), 3.76 (dd, 1H, J = 6.5 and 4.2 Hz), 3.40 (s, 3H), 1.91–1.68 (m, 2H), 1.45–1.00 (br s, 16H), 1.20 (d, 3H, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 200.0 (s), 164.0 (s), 134.8 (d), 132.7 (d), 86.3 (d), 57.9 (q), 45.7 (d), 35.7 (t), 30.1 (t), 28.1 (t), 27.8 (t), 27.8 (t), 27.2 (t), 27.1 (t), 24.0 (t), 21.5 (t), 21.2 (q); MS (ES^+) 296 (M+H), 318 (M+Na), 591 (2M+H), 613 (2M+Na); (ES^-) 294 (M−H), 340 (M+$HCOO^-$).