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3-Hydroxy-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxylates—A new class of HIV-1 integrase inhibitors

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ABSTRACT

A new class of inhibitors of HIV-1 integrase has been optimized to provide selective and highly efficient strand transfer inhibition.

The Acquired Immunodeficiency Syndrome (AIDS) is a major epidemic with more than 33 million infected people worldwide.[1] Its etiological agent has been identified as human immunodeficiency virus type 1 (HIV-1). Current approved therapies target four steps of the HIV life cycle (fusion, reverse transcription, integration and proteolytic maturation).[2] Triple therapy, commonly referred to as HAART (highly active antiretroviral therapy) is the standard for HIV treatment. Unfortunately, despite the great efficacy of HAART long term treatment has many side effects and the selection of mutated viruses leads to the failure of the therapy. Therefore there is a continuous need for new agents with improved properties.

Integrase is an enzyme encoded by the HIV genome and represents a well established target. It catalyses the insertion and integration of the proviral DNA into the genome of the host cell in two steps: 3′-processing (endonucleolytic sequence-specific hydrolysis of the 3′-ends of the viral *c*DNA) and strand transfer (ligation of the viral 3′-OH *c*DNA ends to the phosphate backbone of the host DNA acceptor).[3]

While first generation integrase inhibitors attempted to block the whole assembly process, recent series have been designed to specifically inhibit the strand transfer reaction.[4] Selective inhibition of the integrase activity causes an interruption of the HIV-1 replication cycle and represents an answer to the unmet medical need for new improved treatments.[5] Recently the first integrase inhibitor (raltegravir) was approved in the U.S. and in Europe for treatment of HIV infection.[6]

In a previous article, we reported the discovery of bicyclic pyrimidinones which were created by linking the *N1*-Methyl group of the pyrimidinone scaffold **1**[7] into a saturated cycle as in **2** (Fig. 1).[8]

Herein we describe further studies evolving the 6,7,8,9-tetrahydropyrido[1,2-*a*]pyrimidin-4-one scaffold **2** into a pyrido[1,2-*a*]pyrimidin-4-one scaffold **3** with the aim of removing the benzylic stereocenter.

In order to evaluate the validity of the scaffold, the unsubstituted template **4** was first prepared and tested in the enzymatic assay QUICKIN (QI) (Table 1).

The compound resulted to be a very potent inhibitor of the strand transfer showing an IC_{50} of 22 nM.

Efforts were then directed towards the evaluation of different linker atoms like *N* and *C* for the substituents at position *9* of the bicyclic scaffold.

Amines and amides were tolerated by the enzyme. *N*-methyl amino derivative **5** lost sixfold compared to the unsubstituted scaffold **4** while the *N*-ethyl amino derivative **6** and the benzamide derivative **8** maintained high activity. The *N*,*N*-dimethylamino substituted compound **7** lost threefold.

The substitution of **4** with a methyl group in position *9* led to a twofold gain in enzymatic activity (**9**, IC_{50} = 9 nM). In the light of this result we investigated the homologation of amine and amide derivatives. With the introduction of basic substituents (e.g., **10** and **11**) a nearly 20-fold loss in intrinsic potency was observed.

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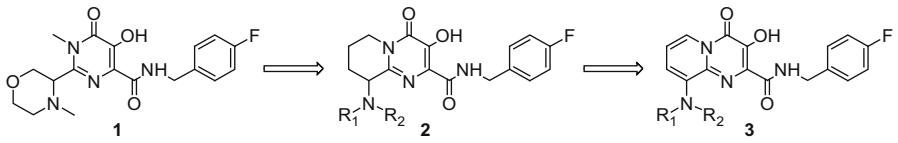
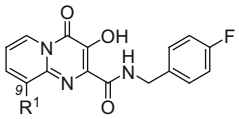
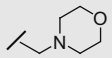
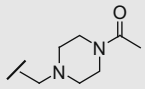


Figure 1. From bicyclic pyrimidinones to fused pyridopyrimidones.

Table 1
In vitro activity



Compound	R^1	QUICKIN IC_{50}[a] (nM)
4	H	22
5	NHMe	130
6	NHEt	36
7	NMe_2	70
8	NHCOPh	18
9	Me	9
10		170
11		185
12	$CH_2NHCOPh$	24

[a] Strand transfer inhibition assay, see Ref. 9.

On the other hand, the homologated benzamide **12** retained activity.

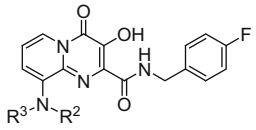
Unfortunately, inhibition in the cell based assay (Spread) was lower than desired even under low serum condition (10% fetal bovine serum). This effect could be due to the combination of suboptimal physical chemical properties, including high lipophilicity. Only **6** exhibited activity (Table 2) less than 1.5 μM.

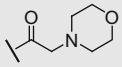
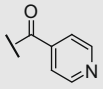
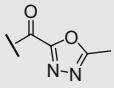
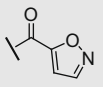
Therefore **6** was chosen as starting point for further SAR and was acylated giving compounds **13**–**19** (Table 2). Based on our previous experience the introduction of amines or small polar heterocycles was beneficial to modulate physico-chemical properties and to improve activity in the cell based assay. Following this strategy some interesting results were obtained.

Upon introduction of *N*,*N*-dimethylglycine a nearly fivefold loss in intrinsic potency was observed (compound **13**). However, the intrinsic activity of **6** could be maintained by substitution with morpholin-4-yl acetamide (**14**). Unfortunately, no improvement in the cell-based assay was achieved.

All compounds **15**–**19** containing a heterocycle fragment showed activity in the strand transfer assay with IC_{50} between 20 and 50 nM and improved activity in the spread assay. The intro-

Table 2
Amide substitution



Compound*	R^2	R^3	QUICKIN IC_{50}[a] (nM)	Spread CIC_{95} [b,c] (nM)	
				10% FBS	50% NHS
6	Et	H	36	1250	>1000
13	Et		185	>1000	>1000
14	Et		23	>1000	>1000
15	Et		50	>1000	>1000
16	Et		21	250	1000
17	Et		50	500	>1000
18	Et		21	250	>1000
19	Et		18	500	>1000

[a] Strand transfer inhibition assay, see Ref. 9.
[b] HIV-1 infection spread inhibition in cell culture, see Ref. 9.
[c] All compounds did not show any toxicity up to 50 μM.

duction of pyridine-2-carboxamide **16** and thiazolecarboxamide **18** resulted in a fivefold improvement in cell-based activity. Interestingly, the position of the pyridine nitrogen does seem to play a role in activity, since the *para*-derivative **15** has a comparatively low activity in the spread assay. This might have to do with the change in basicity of the pyridine nitrogen.

The introduction of an oxadiazole **17** as precedented by raltegravir or of an isoxazole **19** led to a twofold gain in cell-based activity under low-serum conditions.

In other series of HIV integrase inhibitors the presence of an oxalamide had been found to have a positive effect on activity and pharmacological profile.[6a,8] Hence, we decided to study this substitution as well in our new template. All compounds prepared showed comparatively high activity in the enzymatic assay and improved activity in the cell based spread assay in the presence of 10% fetal bovine serum and 50% human serum with respect to **6**. Substitutions were carried out on the two extremities of the oxalamide fragment (Table 3). First *N*,*N*-dimethyl oxalamides differing on the amino group attached to the scaffold (R^2 = Me, Et, *n*Pr, *i*Pr, **20–23**) were prepared and the Et derivative **21** was identified as the most potent compound in the cell-based spread assay at high serum condition, (CIC_{95}: 10% FBS = 62 nM; 50% NHS = 250 nM).

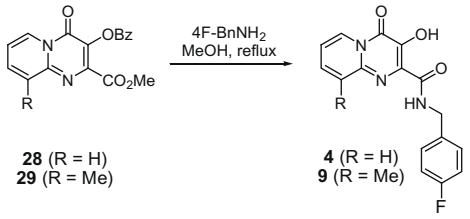
For further evaluation, R^2 = Et was kept fixed and R^4 was modified. Compounds **24–27** maintained a good activity in the enzymatic assay, which for compounds **25–26** translated without a significant shift into the low-serum spread assay. However, this was paired with a pronounced shift (>4-fold) under high serum conditions.

The most potent compound identified was the *N*,*N*-diethylamide **24** which exhibited nanomolar potency in the spread assay at low serum conditions and a twofold shift under high serum conditions (CIC_{95}: 10% FBS = 62 nM; 50% NHS = 125 nM).

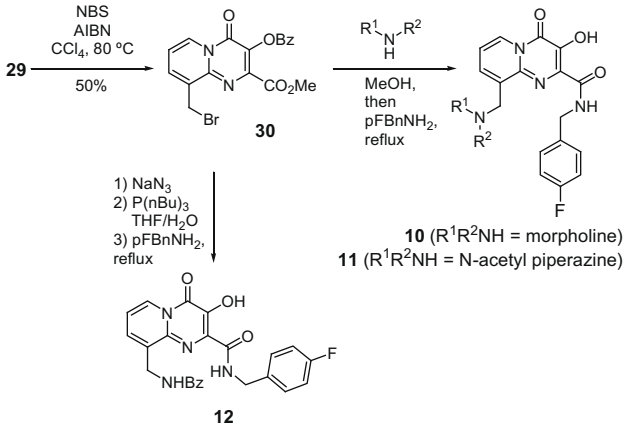
In order to evaluate the PK properties of the series, **21** was further investigated as a representative compound. It was dosed at 3 mg/kg both IV and PO and the rat PK profile was obtained. The compound showed 40% oral bioavailability (F%), plasma exposure of 2.9 μM* h (AUC), plasma half life of 4.5 h ($t_{½}$) and after IV dosing a plasma clearance of 24 ml/min/kg.

The synthesis of the compounds described in Tables 1–3 was based on our recent report on the new pyridopyrimidone scaffold.[10] The methyl esters **28–29** were transformed into the corresponding amides **4** and **9** by standard aminolysis using *para*-fluorobenzyl amine (Scheme 1).

Compound **29** was further elaborated by radical bromination of the methyl group in the benzylic position of the scaffold, fur-

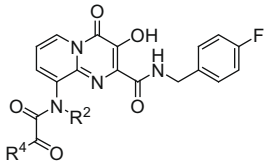


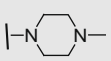
Scheme 1. Benzylamide formation.



Scheme 2. Synthesis of aminomethyl pyrido pyrimidines derivatives.

Table 3
Oxalamide substitution



Compound	R^2	R^4	QUICKIN IC_{50}[a] (nM)	Spread CIC_{95} [b,c] (nM)	
				10% FBS	50%NHS
20	Me	NMe_2	26	125	500
21	Et	NMe_2	36	62	250
22	*n*Pr	NMe_2	18	31	500
23	*i*Pr	NMe_2	21	62	>1000
24	Et	NEt_2	19	62	125
25	Et		25	31	250
26	Et		21	31	1000
27	Et		21	125	500

[a] Strand transfer inhibition assay, see Ref. 9.
[b] HIV-1 infection spread inhibition in cell culture, see Ref. 9.
[c] All compounds did not show any toxicity up to 50 μM.

nishing the bromo derivative **30** in 50% yield. The benzylic bromide **30** was displaced with a variety of secondary amines at room temperature. The corresponding amides **10–11** were obtained by one pot aminolysis with *para*-fluorobenzyl amine (30–40%) (Scheme 2).

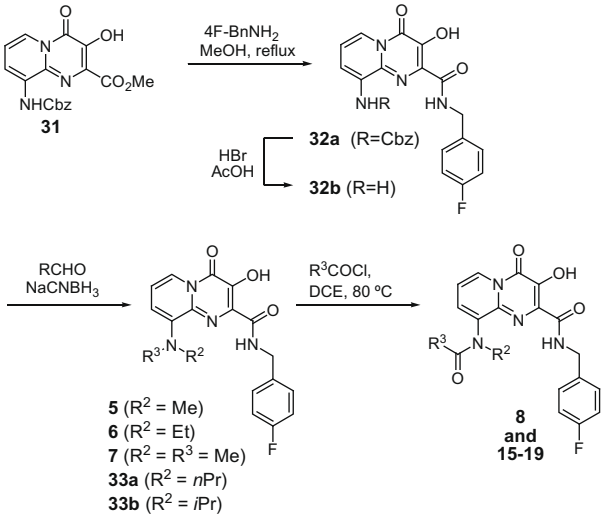
Compound **30** was also reacted under Staudinger conditions with sodium azide to yield, after reduction of the azide, the amino methyl derivative. During this reaction, migration of the benzoate from the 5′-OH to the newly formed amino functionality was observed (14%, compound **12**).

The methyl ester **31**[10] was transformed with para-fluorobenzylamine into the corresponding amide **32a** (Scheme 3).

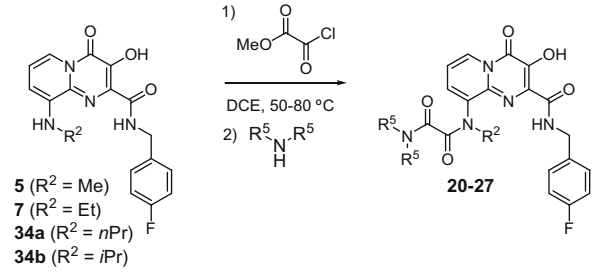
Treatment of **32a** with 37% hydrogen bromide in acetic acid led to the cleavage of the Cbz group liberating the amine **32b** as its hydrobromide salt in quantitative yield. This material was subjected to reductive amination under standard conditions leading to the alkylated amines **5–7** and **33a–b** (30–60%). These amines were then acylated with a series of acid chlorides leading to compounds **8** and **15–19** (46–56%). For the synthesis of the α-amino acid derivatives **13–14**, the acylation was carried out with chloroacetyl chloride, followed by substitution of the resulting alkyl chloride with the corresponding amine (21–25%).

Alternatively, acylation of the monoamines (**5**, **7**, **34**) was carried out by heating them with oxalic acid methylester chloride (Scheme 4). The resulting intermediary oxalic acid methylester amides were transformed in one pot into unsymmetric bis-amides by treatment with different amines, yielding compounds **20–27** (29–56%).[11]

In conclusion, we have designed and studied a new viable alternative bicyclic template of HIV-1-integrase inhibitors, which is characterized by a pyrido[1,2-*a*]pyrimidin-4-one scaffold thus eliminating the presence of any stereocenters in the core of the inhibitor. Various substituents were tolerated at position 9 of the bicyclic scaffold. The presence of an oxalamide was very beneficial for both intrinsic and cellular activity. A number of very potent compounds with nanomolar activity both in the enzymatic and in the cellular spread assay under high serum conditions were identified. The PK profile of the representative compound **21** showed good parameters.



Scheme 3. Synthesis of amino pyrido pyrimidines derivatives.



Scheme 4. Oxalamide synthesis.

Acknowledgements

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References and notes

1. Source: WHO Factsheet HIV. http://www.who.int/hiv/data/2008_global_summary_AIDS_ep.png, accessed September 15th, 2008.
2. (a) Mushawar, I. K. *Perspect. Med. Virol.* **2007**, *13*, 75; (b) Murphy, E.-M.; Jimenez, H. R.; Smith, S. M. *Adv. Pharmacol.* **2008**, *56*, 27.
3. (a) Nair, V. *Rev. Med. Virol.* **2002**, *12*, 179; (b) Young, S. D. *Ann. Rep. Med. Chem.* **2003**, *38*, 173; (c) De Clercq, E. *Exp. Opin. Emerging Drugs* **2005**, *10*, 241.
4. (a) Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. S.; Egbertson, M. S.; Payne, L. P., ; Guare, J. P., Jr.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J., Jr.; Michelson, S. R.; Young, S. D. *J. Med. Chem.* **2003**, *46*, 453; (b) Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M. W.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I.-W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci.* **2004**, *101*, 11233; (c) Petrocchi, A.; Koch, U.; Matassa, V. G.; Pacini, B.; Stillmock, K. A.; Summa, V. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 350; (d) Egbertson, M. S. *Curr. Top. Med. Chem.* **2007**, *7*, 1251.
5. Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Discovery* **2005**, *4*, 236.
6. (a) Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Gonzalez Paz, O.; Hazuda, D. J.; Jones, P.; Kinzel, O.; Laufer, R.; Monteagudo, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, M. V.; Rowley, M. *J. Med. Chem.* **2008**, *51*, 5843; (b) Pace, P.; Rowley, M. *Curr. Opin. Drug Disc. Dev.* **2008**, *11*, 471; (c) Deeks, S. G.; Kar, S.; Gubernick, S. I.; Kirkpatrick, P. *Nature Rev. Drug. Disc.* **2008**, *7*, 117; (d) Sayana, S.; Khanlou, H. *Expert Rev. Anti-Inf. Ther.* **2008**, *6*, 419.
7. Gardelli, C.; Nizi, E.; Muraglia, E.; Crescenzi, B.; Ferrara, M.; Orvieto, F.; Pace, P.; Pescatore, G.; Poma, M.; Rico Ferreira, M. d. R.; Scarpelli, R.; Homnick, C. F.; Ikemoto, N.; Alfieri, A.; Verdirame, M.; Bonelli, F.; Gonzales Paz, O.; Taliani, M.; Monteagudo, E.; Pesci, S.; Laufer, R.; Felock, P.; Stilmock, K. A.; Hazuda, D.; Rowley, M.; Summa, V. *J. Med. Chem.* **2007**, *50*, 4953.
8. (a) Guare, J. P.; Wai, J. S.; Gomez, R. P.; Anthony, N. J.; Jolly, S. M.; Cortes, A. R.; Vacca, J. P.; Felock, P. J.; Stillmock, K. A.; Schleif, W. A.; Moyer, G.; Gabryelski, L. J.; Chen, I.; Hazuda, D. J.; Young, S. D. *Biorg. Med. Chem. Lett.* **2006**, *16*, 2900; (b) Hazuda, D. J.; Young, S. D.; Guare, J. P.; Anthony, N. J.; Gomez, R. P.; Wai, J. S.; Vacca, J. P.; Handt, L.; Motzel, S. L.; Klein, H. J.; Dornadula, G.; Danovich, R. M.; Witmer, M. V.; Wilson, K. A. A.; Tussey, L.; Schleif, W. A.; Gabryelski, L. S.; Jin, L.; Miller, M. D.; Casimiro, D. R.; Emini, E. A.; Shiver, J. W. *Science* **2004**, *305*, 528; (c) Muraglia, E.; Kinzel, O.; Gardelli, C.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Nizi, E.; Orvieto, F.; Pescatore, G.; Laufer, R.; Gonzalez-Paz, O.; Di Marco, A.; Fiore, F.; Monteagudo, E.; Fonsi, M.; Felock, P. J.; Rowley, M.; Summa, V. *J. Med. Chem.* **2008**, *51*, 861.
9. Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. S.; Egbertson, M. S.; Payne, L. P.; Guare, J. P., Jr.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J., Jr.; Michelson, S. R.; Young, S. D. *J. Med. Chem.* **2003**, *46*, 453.
10. Kinzel, O. D.; Donghi, M.; Maguire, C. K.; Muraglia, E.; Pesci, S.; Rowley, M.; Summa, V. *Tetrahedron Lett.* **2008**, *49*, 6556.
11. *Synthetic and brief spectroscopic data on compound* **24**: Step 1: Compound **31**[10] (1.1 mmol) was dissolved in MeOH (0.08 M) and *p*-fluorobenzylamine (2.16 mmol, 2 equiv) was added. The resulting suspension was stirred for 16 h at 80 °C. The solvent was evaporated and the residue washed with HCl 2 M in Et_2O.^{1}HNMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H), 10.44 (s br, 1H), 10.02 (s, 1H), 8.45 (d, J = 7.1 Hz, 1H), 8.24 (d, J = 7.5 Hz, 1H), 7.48–7.38 (m, 6H), 7.18 (m, 3H), 5.29 (s, 2H), 4.61 (s, br, 2H); MS (ES+) *m/z* 463 $(M+H)^+$.Step 2: benzyl (2-{[(4-fluorobenzyl)amino] carbonyl}-3-hydroxy-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-9-yl)carbamate (1.1 mmol) was dissolved in acetic acid (0.07 M) and HBr (30% in acetic acid) was added. The resulting solution was stirred at rt for 2 h. The solvent was evaporated, the residue dissolved several times in toluene and the solvent evaporated. The resulting solid was dissolved in 1,2-dichloroethane-MeOH (1:1, 0.02 M) acetaldehyde (1.1 mmol, 1 equiv) and

sodium cyanoborohydride (1.1 mmol, 1 equiv) were added and the mixture was stirred at rt. After 30 min. the reaction mixture was quenched by the addition of water. The solvent was evaporated and the residue washed with water, (95% yield). ^{1}HNMR (400 MHz, DMSO-d_6) 12.19 (s, 1H), 10.05 (s br, 1H), 8.03 (d, J = 6.9 Hz, 1H), 7.41(m, 2H), 7.20 (m, 2H), 7.04 (m, 1H), 6.5 (d, J = 7.4 Hz, 1H), 4.62 (d, J = 6.0 Hz, 2H), 3.3 (m, 2H), 1.24 (t, J = 6.8 Hz, 3H); MS (ES+) m/z 356 $(M+H)^+$.Step 3: Compound *N*-(4fluorobenzyl)-3-hydroxy 9(ethylamino)-4-oxo-4*H*-pyrido[1,2-a]pyrimidine-2-carboxamide (0.17 mmol) was dissolved in 1,2-dichloroethane (0.02 M) and methyl chloro(oxo)acetate (0.34 mmol, 2 equiv) was added. The resulting solution was heated to 80 °C and stirred for 1 h. The solvent was evaporated; the residue dissolved in MeOH (0.02 M) and diethylamine (0.17 mmol, 1 equiv) was added. The resulting solution was heated to 50 °C and stirred for 1 h. The solvent was evaporated and the residue dissolved in acetonitrile and purified by RP-HPLC (stationary phase: Symmetry C_{18}, 5 μm, 19 × 300 mm. Mobile phase: acetonitrile/H_2O buffered with 0.1% TFA). The fractions were combined and lyophilised to afford the title compounds as yellow powder (23% yield). The ^{1}H NMR spectrum shows the presence of two conformers in a ratio of 3:2.*Compound* **24**: ^{1}H NMR (400 MHz, CD_3CN) δ 12.48 (s, br, 0.6H), 12.08 (s, br, 0.4H), 9.63 (s, br, 0.4H), 8.74 (d, J = 7.41 Hz, 0.4H), 8.59 (s, br, 0.6H), 7.64 (d, J = 6.9 Hz, 0.4H), 7.59 (d, J = 6.9 Hz, 0.6H), 7.48 (t, br, 0.8H), 7.42 (t, br, 1.2H), 7.13–7.06 (m, 3H), 4.68–4.56 (m, 2H), 4.43 (m, 0.6H), 3.5–3.42 (m, 4H), 3.23 (m, 0.4H), 2.91–2.83 (m, 1H), 1.20 (t, J = 6.9 Hz, 1.5H), 1.11 (t, J = 6.5 Hz, 1.5H), 0.9 (t, J = 6.8 Hz, 1.5H), 0.58 (t, J = 6.9 Hz, 1.5H). MS (ES+) m/z 484 $(M+H)^+$.