Tetrahedron Letters 50 (2009) 2150–2153

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Enzymatic formation of unnatural novel polyketide scaffolds by plant-specific type III polyketide synthase

She-Po Shi [a], Hiroyuki Morita [a], Kiyofumi Wanibuchi [a], Hiroshi Noguchi [a], Ikuro Abe [a,b,*]

[a] School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan
[b] PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan

ARTICLE INFO

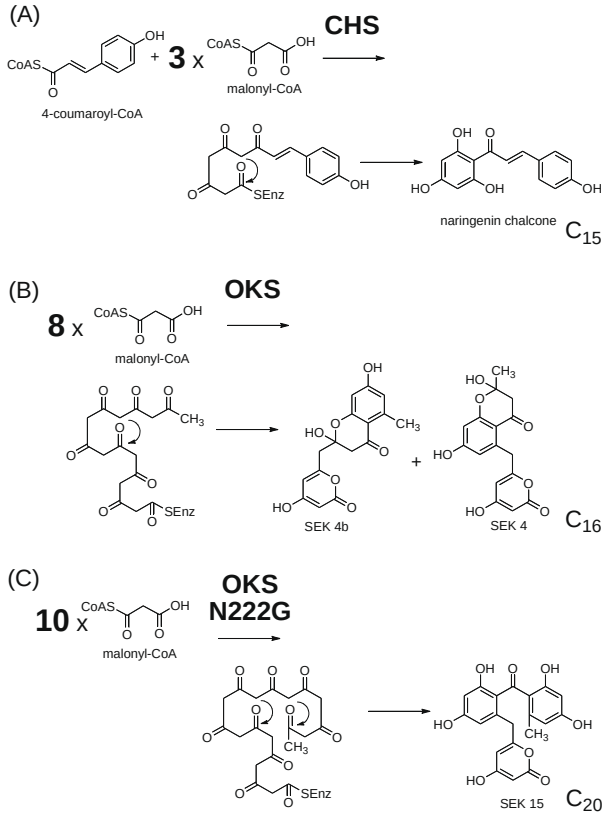
Article history:
Received 10 January 2009
Revised 19 February 2009
Accepted 23 February 2009
Available online 26 February 2009

ABSTRACT

The catalytic potential of octaketide synthase (OKS), a plant-specific type III polyketide synthase (PKS) from *Aloe arborescens*, was investigated by phenylacetyl-CoA and benzoyl-CoA as starter substrates. As a result, a novel C_{16} pentaketide coumarin was produced from phenylacetyl-CoA, whereas benzoyl-CoA was not a good substrate of OKS. Remarkably, a structure-guided OKS N222G mutant dramatically extended the product chain length to yield four novel polyketides including C_{22} aromatic octaketides from the C_6–C_2 phenylacetyl starter, as well as a novel C_{19} heptaketide benzophenone from the C_6–C_1 benzoyl starter.

The chalcone synthase (CHS) superfamily of type III polyketide synthases (PKSs) catalyze iterative decarboxylative condensations of malonyl-CoA with a CoA-linked thioester to perform sequential C–C bonds formation, which generate a variety of plant secondary metabolites with remarkable structural diversity and biological activities.[1] A typical example is CHS that catalyzes sequential condensation of 4-coumaroyl-CoA with three molecules of malonyl-CoA to produce a tetraketide naringenin chalcone (Scheme 1A).[1,2] The remarkable functional diversity of the CHS-superfamily enzymes derives from the differences of selection of the starter molecules, the number of the malonyl-CoA condensations, and the mechanisms of cyclization reactions. Despite the fact that the broad substrate tolerance and catalytic potential of type III PKSs have been extensively investigated,[3,4] those compounds that have been identified and isolated represent only the tip of the iceberg and there is a wealth still to be mined.

Octaketide synthase (OKS) from *Aloe arborescens* is a plant-specific type III PKS catalyzing sequential condensations of eight molecules of malonyl-CoA to yield SEK4 and SEK4b (Scheme 1B).[5] The C_{16} aromatic octaketides are known to be the shunt products of the minimal type II PKS for actinorhodin (*act* from *Streptomyces coelicolor*).[6] Like other type III PKSs, *A. arborescens* OKS exhibits unusually broad, promiscuous substrate specificities. The enzyme accepts disparate starter molecules, both aromatic and aliphatic CoA thioesters with different chain length, and efficiently performs sequential condensation and cyclization reactions to produce a series of chemically and structurally different unnatural polyketides.[5a,5e] Here we now report a novel C_{16} pentaketide coumarin produced by *A. arborescens* OKS as well as four unnatural novel



Scheme 1. Enzymatic formation of (A) naringenin chalcone by CHS, (B) SEK4/SEK4b by OKS, and (C) SEK15 by OKS N222G mutant.

* Corresponding author. Tel./fax: +81 54 264 5662.
E-mail address: abei@u-shizuoka-ken.ac.jp (I. Abe).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.02.170

heptaketides and octaketides generated by a structure-guided OKS N222G mutant from phenylacetyl-CoA as a starter substrate. In addition, a novel C_{19} heptaketide benzophenone produced by the OKS N222G mutant from benzoyl-CoA is also described.

When recombinant wild-type *A. arborescens* OKS was incubated with phenylacetyl-CoA and malonyl-CoA as substrates, the enzyme efficiently accepted phenylacetyl-CoA as a starter to produce a novel C_{16} pentaketide (**1**) (2.3 mg, 22% yield) with a parent ion peak $[M+H]^+$ at *m/z* 269 on LC-ESIMS (Fig. 1A).[7] By carefully analyzing its NMR data and comparing these to those of the known natural products such as 2,7-dihydroxy-5-methylchromone and 4,7-dihydroxy-5-methylcoumarin,[5a,8] the structure of the pentaketide product was unambiguously elucidated as 5-benzyl-4,7-dihydroxy-coumarin (**1**) (Scheme 2A). All the protons and carbons were completely assigned with the help of 2D NMR including HMQC and HMBC.[9] Thus, sequential condensation of the C_6–C_2 phenylacetyl starter with four molecules of malonyl-CoA afforded the novel C_{16} pentaketide coumarin (Scheme 2A).

Coumarins as a big family of plant secondary metabolites are widely distributed among many plant families, including Apiacea, Asteraceae, Fabiaceae, Rutaceae, Saxifragaceae, Solanaceae, and Umbelliferae.[10] For the biosynthesis of coumarins, it is commonly accepted that they originate from the general phenylpropanoid pathway, and the pivotal step is the hydroxylation at the *ortho*-position of cinnamates which is generally presumed to be catalyzed by a P450 enzyme.[11] The present result for the first time clearly demonstrated that type III PKSs can also generate the coumarin scaffold. On the other hand, base-catalyzed chemical cyclization

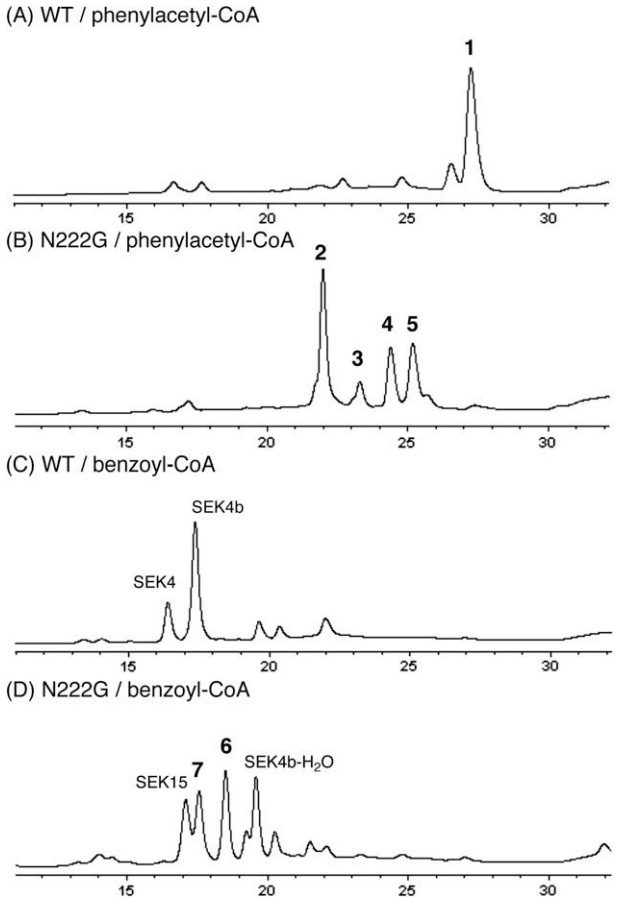
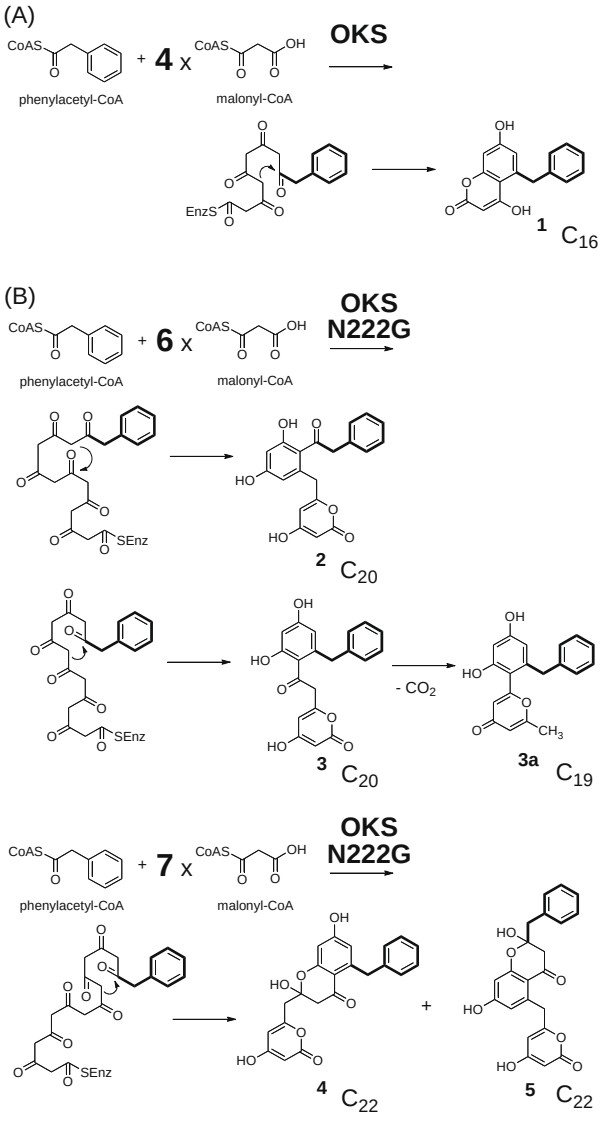


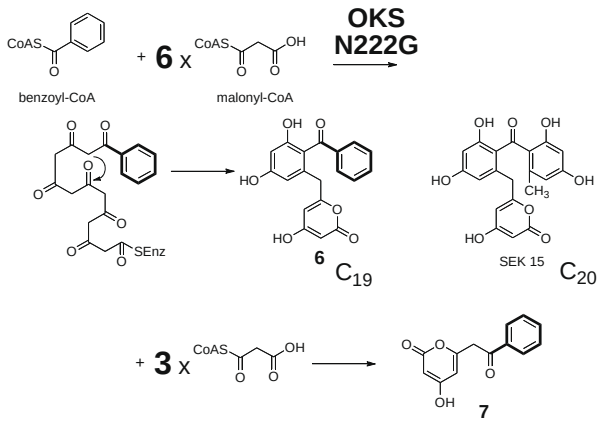
Figure 1. HPLC elution profiles (A_{280}) of enzyme reaction products from (A) phenylacetyl-CoA and malonyl-CoA by OKS wild-type, (B) phenylacetyl-CoA and malonyl-CoA by OKS N222G mutant, (C) benzoyl-CoA and malonyl-CoA by OKS wild-type, and (D) benzoyl-CoA and malonyl-CoA by OKS N222G mutant.



Scheme 2. Enzymatic formation of (A) C_{16} pentaketide coumarin by OKS, and (B) C_{20} heptaketides and C_{22} octaketides by OKS N222G mutant (from the phenylacetyl starter).

of 3,5,7,9-tetraoxo-9-phenylnonanoic acid into 5-phenyl-4,7-dihydroxy-coumarin via a resorcinol carboxylic acid has been reported.[12]

Previously we have reported that a structure-guided OKS N222G mutant, in which the active-site cavity was expanded by a large to small substitution of N222 at the bottom of the polyketide chain elongation tunnel, efficiently produces a C_{20} decaketide benzophenone, SEK15, by sequential condensations of 10 molecules of malonyl-CoA (Scheme 1C).[5e] Remarkably, incubation of the N222G mutant with phenylacetyl-CoA and malonyl-CoA under the same condition afforded four novel products (**2**–**5**) with extended chain length (Fig. 1B). The LC-ESIMS spectra indicated that products **2** (1.3 mg, 13% yield) and **3** (0.5 mg, 5% yield) possessed the same parent ion peak $[M+H]^+$ at *m/z* 353 on LC-ESIMS, while products **4** (0.9 mg, 10% yield) and **5** (0.9 mg, 10% yield) shared $[M+H]^+$ at *m/z* 395. These results suggested that **2** and **3** were C_{20} heptaketides produced by condensation of six molecules of malonyl-CoA with phenylacetyl-CoA, while **4** and **5** were C_{22} octaketides produced by condensation of seven molecules of malonyl-CoA with phenylacetyl-CoA (Scheme 2B). The structures of **2**, **4** (benzyl-SEK4b), and **5** (benzyl-SEK4) were unambiguously elucidated; all



Scheme 3. Enzymatic formation of a C_{19} heptaketide benzophenone and a tetraketide pyrone by OKS N222G mutant (from the benzoyl starter).

protons and carbons were completely assigned by NMR (^{1}H and ^{13}C NMR, HMQC, and HMBC) spectroscopy.[13–15]

For the product **3,** its ESIMS spectrum indicated loss of a typical ion peak 126, which is a characteristic fragment for the 4-hydroxy-6-methylpyran-2-oxo moiety. Unfortunately, the structural elucidation of **3** by NMR failed because this compound is very unstable, and spontaneously and completely changed into a C_{19} heptaketide (**3a**) (0.5 mg, 5% yield), whose structure was unambiguously elucidated by NMR (^{1}H and ^{13}C NMR, HMQC, and HMBC) and MS spectroscopy.[16] Considering the ESIMS data and biogenetic reasoning, it is likely that **3a** was produced from the putative intermediate **3** via opening of the terminal pyrone ring, decarboxylation, and recyclization (Scheme 2B). Similar decarboxylative conversion of a keto 2-pyrone to a 4-pyrone has been also reported in a literature.[12]

In contrast, the smaller C_6–C_1 benzoyl-CoA did not fit well into the active-site of wild-type *A. arborescens* OKS. When the enzyme was incubated with benzoyl-CoA and malonyl-CoA as substrates, most of the enzyme reactions were initiated by malonyl-CoA, and the octaketides SEK4 and SEK4b were obtained as dominant products (Fig. 1C). On the other hand, however, the structure-guided N222G mutant with the expanded active-site cavity accepted the benzoyl starter and carried out six condensations with malonyl-CoA to produce a novel C_{19} heptaketide benzophenone (**6**) (0.8 mg, 8% yield) as well as a tetraketide 4-hydroxy-6-(2-oxo-2-phenylethyl)-2-pyrone (**7**)[3b] (0.6 mg, 7% yield) (Fig. 1D and Scheme 3). The structures were unambiguously established by NMR (^{1}H and ^{13}C NMR, HMQC, and HMBC) and MS spectroscopy.[17] Here it should be noted that the structure of the C_{19} heptaketide **6** showed close similarity to that of the C_{20} decaketide SEK15 produced from 10 molecules of malonyl-CoA by the N222G mutant (Scheme 1C).

In summary, the present work describes the enzymatic formation of six novel polyketides from phenylacetyl-CoA and benzoyl-CoA by a plant-specific type III PKS. It is remarkable that wild-type OKS and the structure-guided N222G mutant afforded significantly different product pattern; the wild-type enzyme produced C_{16} pentaketide coumarin, whereas the point mutant dramatically extended the product chain length up to the C_{22} octaketides which are currently the longest polyketides generated by the structurally simple type III PKSs. Further, the C_6–C_2 phenylacetyl starter better fits into the active-site of the enzymes and more efficiently initiated the sequential condensation reactions than the C_6–C_1 benzoyl starter. In all the cases, the enzymes catalyzed the chain elongation and the first aromatic ring formation at the middle of the polyketide intermediates as in the case of the production of SEK4/SEK4b and SEK15. Formation of the terminal α-pyrone ring could be an important process for the release of the polyketide products from the thioester-linked active-site cysteine.[5e] The results suggested that manipulation of the enzyme reactions by combination of the precursor-directed biosynthesis and structure-guided engineering of the enzyme would lead to further production of unnatural novel polyketide scaffolds.

Acknowledgments

This work was supported by the PRESTO program from Japan Science and Technology Agency, Grant-in-Aid scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and Research Grants from the Astellas Foundation for Research on Metabolic Disorders, and the Uehara Memorial Foundation, Japan. S.-P.S. and K.W. are recipients of the JSPS Postdoctoral Fellowship for Foreign Researchers (no. P07048) and the JSPS Fellowship for Young Scientists (no. 207112), respectively.

Supplementary data

Supplementary data (experimental details and a complete set of NMR data and charts are provided) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.02.170.

References and notes

1. For recent reviews, see: (a) Austin, M. B.; Noel, J. P. *Nat. Prod. Rep.* **2003**, *20*, 79–110; (b) Schröder, J.. In *Comprehensive Natural Products Chemistry*; Elsevier: Oxford, 1999; Vol. 2, pp 749–771.
2. (a) Ferrer, J. L.; Jez, J. M.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. *Nat. Struct. Biol.* **1999**, *6*, 775–784; (b) Jez, J. M.; Ferrer, J. L.; Bowman, M. E.; Dixon, R. A.; Noel, J. P. *Biochemistry* **2000**, *39*, 890–902.
3. (a) Abe, I.; Morita, H.; Nomura, A.; Noguchi, H. *J. Am. Chem. Soc.* **2000**, *122*, 11242–11243; (b) Morita, H.; Takahashi, Y.; Noguchi, H.; Abe, I. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 190–195; (c) Morita, H.; Noguchi, H.; Schröder, J.; Abe, I. *Eur. J. Biochem.* **2001**, *268*, 3759–3766; (d) Abe, I.; Takahashi, Y.; Noguchi, H. *Org. Lett.* **2002**, *4*, 3623–3626; (e) Abe, I.; Takahashi, Y.; Lou, W.; Noguchi, H. *Org. Lett.* **2003**, *5*, 1277–1280; (f) Abe, I.; Sano, Y.; Takahashi, Y.; Noguchi, H. *J. Biol. Chem.* **2003**, *278*, 25218–25226; (g) Abe, I.; Watanabe, T.; Noguchi, H. *Phytochemistry* **2004**, *65*, 2447–2453; (h) Oguro, S.; Akashi, T.; Ayabe, S.; Noguchi, H.; Abe, I. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 561–567; (i) Abe, T.; Noma, H.; Noguchi, H.; Abe, I. *Tetrahedron Lett.* **2006**, *47*, 8727–8730; (j) Abe, I.; Abe, T.; Wanibuchi, K.; Noguchi, H. *Org. Lett.* **2006**, *8*, 6063–6065; (k) Abe, I.; Watanabe, T.; Lou, W.; Noguchi, H. *FEBS J.* **2006**, *273*, 208–218; (l) Wanibuchi, K.; Zhang, P.; Abe, T.; Morita, H.; Kohno, T.; Chen, G.; Noguchi, H.; Abe, I. *FEBS J.* **2007**, *274*, 1073–1082.
4. (a) Schüz, R.; Heller, W.; Hahlbrock, K. *J. Biol. Chem.* **1983**, *258*, 6730–6734; (b) Zuurbier, K. W. M.; Leser, J.; Berger, T.; Hofte, A. J. P.; Schröder, G.; Verpoorte, R.; Schröder, J. *Phytochemistry* **1998**, *49*, 1945–1951; (c) Jez, J. M.; Bowman, M. E.; Noel, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5319–5324; (d) Samappito, S.; Page, J.; Schmidt, J.; De-Eknamkul, W.; Kutchan, T. M. *Planta* **2002**, *216*, 64–71; (e) Samappito, S.; Page, J. E.; Schmidt, J.; De-Eknamkul, W.; Kutchan, T. M. *Phytochemistry* **2003**, *62*, 313–323; (f) Springob, K.; Samappito, S.; Jindaprasert, A.; Schmidt, J.; Page, J. E.; De-Eknamkul, W.; Kutchan, T. M. *FEBS J.* **2007**, *274*, 406–417.
5. (a) Abe, I.; Oguro, S.; Utsumi, Y.; Sano, Y.; Noguchi, H. *J. Am. Chem. Soc.* **2005**, *127*, 12709–12716; (b) Morita, H.; Kondo, S.; Kato, R.; Wanibuchi, K.; Noguchi, H.; Sugio, S.; Abe, I.; Kohno, T. *Acta Crystallogr., Sect. F* **2007**, *63*, 947–949; (c) Abe, I. In *ACS Symposium Series* 955; American Chemical Society: Washington, DC, 2007, pp 109–127.; (d) Abe, I. *Chem. Pharm. Bull.* **2008**, *56*, 1505–1514; (e) Shi, S.-P.; Wanibuchi, K.; Morita, H.; Endo, K.; Noguchi, H.; Abe, I. *Org. Lett.* **2009**, *11*, 551–554.
6. (a) Fu, H.; Ebert-Khosla, S.; Hopwood, D. A.; Khosla, C. *J. Am. Chem. Soc.* **1994**, *116*, 4166–4170; (b) Fu, H.; Hopwood, D. A.; Khosla, C. *Chem. Biol.* **1994**, *1*, 205–210.
7. Steady state kinetic analysis revealed that K_M = 91.6 μM and k_{cat} = 0.022 min^{-1} for phenylacetyl-CoA for the C_{16} coumarin forming activity.
8. Hűttel, W.; Műller, M. *ChemBioChem* **2007**, *8*, 521–529.
9. *Product* ***1***: LC-ESIMS: R_t = 27.5 min, *m/z* 269 [M+H]$^+$. UV: λ_{max} 312 nm; ^{1}H NMR (400 MHz, CD_3OH): δ 7.10–7.26 (m, 5H), 6.61 (br s, 1H), 6.56 (br s, 1H), 5.38 (s, 1H), 4.46 (s, 2H). ^{13}C NMR (100 MHz, CD_3OH): δ 171.7, 166.4, 162.5, 158.9, 143.2, 142.2, 129.6 (2×), 129.2 (2×), 126.8, 117.8, 107.9, 102.2, 89.0, 41.4. HRMS (FAB, positive): found for $[C_{16}H_{13}O_4]^+$ 269.0808; calcd 269.0814.
10. (a) Keating, G. J.; O'Kennedy, R. In *Coumarins: Biology, Applications and Mode of Action*; O'Kennedy, R., Thornes, R. D., Eds.; John Wiley & Sons: Chichester, 1997; pp 23–66; (b) Zobel, A. M. In *Phytochemistry of Fruits and Vegetables*; Tomas-Barberan, F. A. A., Robins, R. J., Eds.; Clarendon Press: Oxford, 1997; pp 173–203.
11. Bourgaud, F.; Hehn, A.; Larbat, R.; Doerper, S.; Gontier, E.; Kellner, S.; Matern, U. *Phytochem. Rev.* **2006**, *5*, 293–308.

12. Harris, T. M.; Harris, C. M. *Pure Appl. Chem.* **1986**, *58*, 283–294.
13. *Product* ***2***: LC-ESIMS: R_t = 22.1 min, *m/z* 353 $[M+H]^+$. UV: λ_{max} 286 nm; ^1H NMR (400 MHz, DMSO-d_6): δ 7.15–7.24 (m, 5H), 6.32 (br s, 1H), 6.16 (br s, 1H), 5.64 (br s, 1H), 5.12 (br s, 1H), 4.13 (s, 2H), 3.51 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 202.9, 173.2, 164.7, 163.9, 159.6, 157.4, 135.9, 135.4, 129.8 (2×), 128.0 (2×), 126.3, 120.0, 109.4, 101.6, 100.8, 88.1, 50.2, 36.3. HRMS (FAB, positive): found for $[C_{20}H_{17}O_6]^+$ 353.1040; calcd 353.1025.
14. *Product* ***4***: LC-ESIMS: R_t = 24.6 min, *m/z* 395 $[M+H]^+$; UV: λ_{max} 236, 280 nm; ^1H NMR (400 MHz, CD_3OH): δ 7.10–7.18 (m, 5H), 6.20 (br s, 1H), 6.12 (br s, 2H), 5.34 (br s, 1H), 4.31 (d, 1H, *J* = 13.6 Hz), 4.30 (d, 1H, *J* = 13.6 Hz), 3.04 (d, 2H, *J* = 14.8 Hz), 2.97 (d, 1H, *J* = 16.0 Hz), 2.66 (d, 1H, *J* = 16.0 Hz). ^{13}C NMR (100 MHz, CD_3OH): δ 192.6, 173.0, 168.0, 165.0, 163.1, 162.1, 147.4, 141.7, 130.1 (2×), 129.1 (2×), 126.7, 114.2, 112.8, 105.2, 103.3, 101.1, 90.5, 49.9, 45.6, 40.8. HRMS (FAB, positive): found for $[C_{22}H_{19}O_7]^+$ 395.1122; calcd 395.1131.
15. *Product* ***5***: LC-ESIMS: R_t = 25.4 min, *m/z* 395 $[M+H]^+$; UV: λ_{max} 236, 279 nm; ^1H NMR (400 MHz, CD_3OH): δ 7.29 (m, 5H), 6.35 (br s, 2H), 5.63 (br s, 1H), 5.22 (br s, 1H), 4.20 (d, 1H, *J* = 16.0 Hz), 4.10 (d, 1H, *J* = 16.0 Hz), 3.23 (d, 1H, *J* = 13.6 Hz), 3.11 (d, 1H, *J* = 13.6 Hz), 2.75 (d, 1H, *J* = 16.0 Hz), 2.44 (d, 1H, *J* = 16.0 Hz). ^{13}C NMR (100 MHz, CD_3OH): δ 193.3, 175.2, 166.9, 165.2 (2×), 163.8, 140.1, 136.7, 131.6 (2×), 129.1 (2×), 127.8, 114.9, 112.8, 104.4, 103.1, 102.6, 89.4, 49.6, 47.6, 39.2. HRMS (FAB, positive): found for $[C_{22}H_{18}O_7Na]^+$ 417.0943; calcd 417.0950.
16. *Product* ***3a***: UV: λ_{max} 296 nm; ^1H NMR (400 MHz, CD_3OD): δ 7.17–7.24 (m, 5H), 6.68 (br s, 1H), 6.60 (br s, 1H), 6.23 (br s, 1H), 6.08 (br s, 1H), 4.62 (s, 2H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 178.4, 168.6, 164.9, 163.3, 161.6, 145.9, 140.4, 130.1 (2×), 129.3 (2×), 126.9, 118.5, 117.1, 113.7, 111.1, 102.2, 40.8, 21.6. HRMS (FAB, positive): found for $[C_{19}H_{17}O_4]^+$ 309.1129; calcd 309.1127.
17. *Product* ***6***: LC-ESIMS: R_t = 18.6 min, *m/z* 339 $[M+H]^+$. UV: λ_{max} 258 nm; ^1H NMR (400 MHz, CD_3OH): δ 7.73 (m, 2H), 7.53 (m, 1H), 7.42 (m, 2H), 6.37 (br s, 1H), 6.31 (br s, 1H), 5.69 (br s, 1H), 5.02 (br s, 1H), 3.64 (s, 2H). ^{13}C NMR (100 MHz, CD_3OH): δ 200.1, 173.1, 167.1, 165.5, 161.2, 158.5, 140.0, 137.1, 134.2, 130.4 (2×), 129.4 (2×), 120.2, 110.7, 103.6, 102.7, 89.6, 38.2. HRMS (FAB, positive): found for $[C_{19}H_{14}O_6Na]^+$ 361.0691; calcd 361.0688.