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Synthesis of 1,1-dioxopenicillanoyloxymethyl 6-[D-α-(benzylideneaminophenylacetamido)]penicillanate and analogs. New intermediates in the preparation of sultamicillin

Carlos del Pozo,[a,*] Eduardo Alonso,[a] Fernando López-Ortiz,[b] Félix Fernández-Marí,[c] Miguel Bayod[c] and Javier González[a,*]

[a]*Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, 33071 Oviedo, Spain*
[b]*Área de Química Orgánica, Facultad de Ciencias Experimentales, Universidad de Almería, Carretera de Sacramento, 04120 Almeria, Spain*
[c]*Asturpharma, Polígono Industrial de Silvota, Parcela 22B-23, 33192 Llanera, Asturias, Spain*

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Abstract—The synthesis of benzylidene imines of sultamicillin and analogs **1** is described. These new compounds showed high stability and were prepared under very mild conditions and high yields. Furthermore, they are convenient starting materials for the preparation of sultamicillin **5** (important prodrug of ampicillin and sulbactam) as its base form, by deprotection of the amino group with Girard reagents.

1. Introduction

Penicillins and cephalosporins have proven to be safe and highly effective drugs in the treatment of infectious diseases for over 60 years. However, this widespread usage explains the appearance and increase of bacterial resistances towards conventional penicillins.

The problem of the bacterial resistance to β-lactam antibiotics is becoming a world-health challenge and the development of new types of these antibiotics is now an important priority in the pharmaceutical research,[1] and many efforts are being made to improve the therapeutic properties of these products (for example, the search of new analogs with improved antibacterial activity). Thus, chemists have developed several chemical means to overcome bacterial resistances,[2] stability problems and pharmacokinetic defects (such as poor oral absorption).

In this sense, the use of prodrug strategies as a method to avoid oral absorption problems has been carried out profusely in the case of penicillins.[3] Ampicillin is the most widely used broad-spectrum antibacterial penicillin in clinical use, and several oral prodrug forms of this antibiotic have been marketed.[4] Sultamicillin (CAS Registry Number 76497-13-7) is one of the aforementioned ampicillin prodrugs, bearing a double ester of methanodiol with sulbactam[5] and ampicillin (this namely double ester of a geminal diol is a structurally common feature of these effective prodrugs).[6] This compound incorporates, in the same molecule, the antibiotic and the β-lactamase inhibitor, and is rapidly hydrolyzed in blood, releasing the active species. Furthermore, it allows simultaneously the oral administration of both sulbactam and ampicillin, overcoming the poor oral absorption showed by the former.

The synthesis of this molecule has been described in several patents.[7] Fig. 1 summarizes the commonly used synthetic strategy. It involves *O*-alkylation of ampicillin itself **2** or *N*-protected ampicillin **3** with a halomethyl ester of sulbactam **4** to afford the methanodiol ester **5** of both, ampicillin and sulbactam (after removal of the nitrogen protecting group when compound **3** is used). Further improvements in the synthesis have also been described. However, all of them use the above mentioned strategy, introducing small changes in the process.[7c,8]

The nature of the *N*-protecting group of ampicillin in the preparation of sultamicillin has been limited to a few groups including azide, benzyloxycarbonyl, triphenylmethyl, 1-methoxycarbonylpropen-2-yl, 1-*N*,*N*′-dimethylaminocarbonylpropen-2-yl and some other heterocyclic groups.[7c] The choice of a protecting group may have a large impact on the quality of the final drug. In fact, the currently available methods to prepare sultamicillin suffer from the same

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* Corresponding authors. Tel.: +985-103456; fax: +985-103446; e-mail: fjgf@sauron.quimica.uniovi.es

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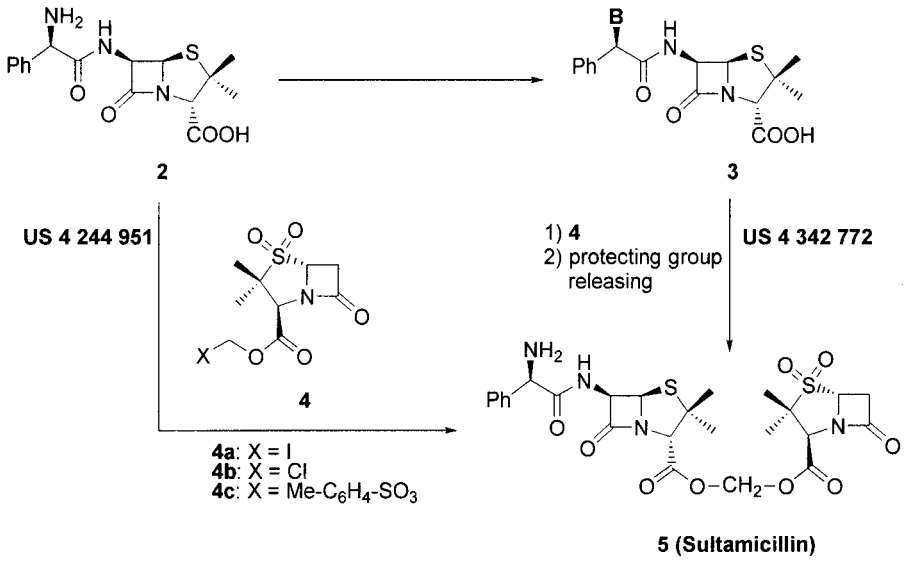


Figure 1.

practical difficulties:

1. Unstability of the *N*-protecting group against the coupling conditions between the protected ampicillin and the halomethyl ester of sulbactam.
2. Unstability of sultamicillin in the experimental conditions used to release the protecting group and in the purification of the final product.

The use of an imino group to protect the nitrogen of ampicillin in the synthesis of sultamicillin had never been reported in the literature. However, the ampicillin derived antibiotic metampicillin, which contains a methylidenimine group, and its association with sulbactam leading to a sultamicillin analog, has already been described.[9] Imines as *N*-protecting groups have also been used to couple sulbactam with other penicillins and cephalosporins, showing high stability in the coupling conditions.

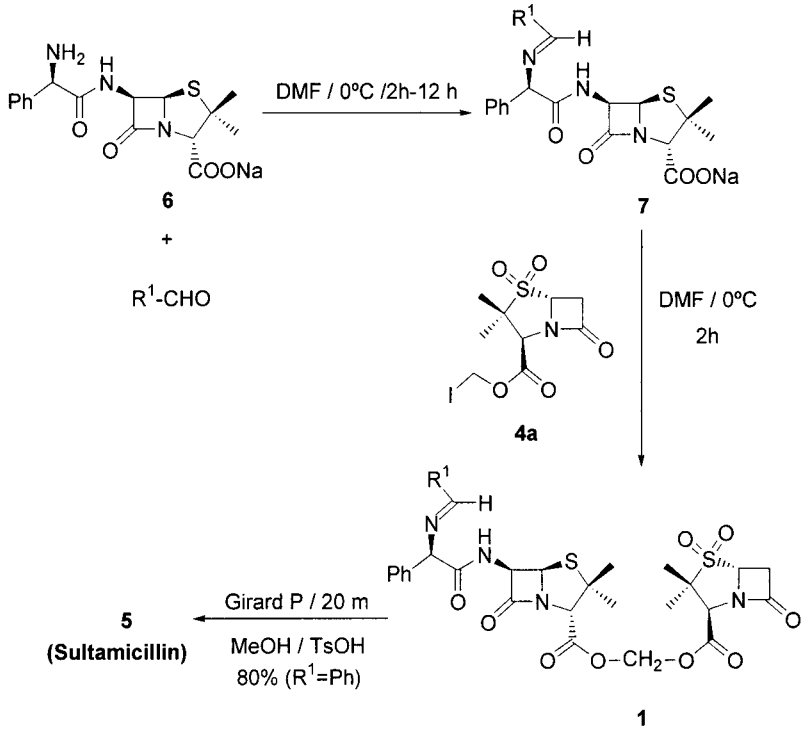
In this paper we report the use of imines in the sultamicillin field to protect the nitrogen of ampicillin during the coupling reaction with sulbactam.[10] The imines were obtained in excellent yields and showed high stability under the experimental conditions of the coupling process. In addition, protecting group release can be carried out under mild conditions and good yields. Therefore, the imines described in this paper are interesting intermediates for the efficient preparation of pure sultamicillin.

2. Results and discussion

The reaction of ampicillin with several aldehydes affording the corresponding imines has been previously studied.[11] The imine acted as a protecting group of the ampicillin amino group[12] and the esterification of its carboxyl functionality allowed entry to methanodiol derivatives, a strategy that, to the best of our knowledge, has never been used in the preparation of sultamicillin.

In an optimized procedure 1.2 equiv. of benzaldehyde was added to a solution of ampicillin sodium salt **6** in DMF (0.25 M solution) at 0°C, and the resultant reaction mixture was stirred for two hours. Subsequently 1.2 equiv. of iodomethyl 1,1-dioxopenicillanate **4a** was added, and aqueous work up led to the benzylidenimine of sultamicillin **1a** in high yield. The reaction was further extended to the preparation of several different sultamicillin imines **1** by using 2-, 3- or 4-nitro or halogen substituted benzaldehydes, heteroaromatic aldehydes and also aliphatic aldehydes (Scheme 1, Table 1).

The structure of these new imine derivatives was unambiguously determined by a combination of mass spectrometry and NMR experiments. In the following we outline the key steps of the assignment procedure of compound **1a**. The high resolution fast atom bombardment mass spectrum (FAB-HRMS) showed the quasimolecular ion at 683 corresponding to the molecular formula $C_{32}H_{34}N_4O_9S_2$ (M+1: 683.1845) expected for **1a**. The presence of the imine moiety was deduced from the singlet at δ 8.3 ppm in the ^{1}H NMR spectrum, easily assigned to the iminic proton. The iminic carbon appeared as the most deshielded signal in the ^{13}C DEPT-135 spectrum and this assignment was confirmed through the 2D HMQC spectrum by observing the cross peak of this carbon atom with the proton at 8.3 ppm. Using these two assignments as starting point, the connectivity of the whole molecule could be deduced and the ^{1}H and ^{13}C NMR spectra fully assigned. Thus, the iminic proton showed a strong cross peak in the 2D HMBC spectrum with the methine carbon (δ 75.96 ppm) of the phenylglycine moiety of the side chain of the ampicillin fragment. The ^{1}H chemical shift of this CH was identified at δ 5.04 ppm in the HMQC spectrum. This CH afforded a new entry point to continue the assignment of the ^{1}H and



Scheme 1.

Table 1. Preparation of sultamicillin imines **1**

1	R^1	Yield (%)[a]	δ (iminic CH, ^{1}H, ppm)[b]	δ (iminic CH, ^{13}C, ppm)[b]
a	Ph	91	8.30	163.5
b	*o*-NO_2–C_6H_4	74	8.75	159.8
c	*m*-NO_2–C_6H_4	68	8.38	161.0
d	*o*-Cl–C_6H_4	71	8.74	160.4
e	*p*-Cl–C_6H_4	84	8.20	162.0
f	2-Furyl	89	8.07	151.7
g	3-Pyridyl	94	8.32	161.0
h	*tert*-Butyl	81	7.61	175.2

[a] Yield of isolated product after purification.
[b] Selected data were recorded on a Bruker AC-300.

^{13}C NMR spectra by repeating the alternating analysis of the HMQC and HMBC spectra. The connection between the ampicillin and sulbactam molecular fragments was established through the methylene group characterized by δ(H) 5.86 and 5.94 ppm, $^2J_{HH}$=8.6 Hz), and δ(C) 80.58 ppm. A detailed discussion of the structure assignment will be published elsewhere.[13]

It is noteworthy the high stability showed by these compounds. Thus, they are stable to flash chromatography, they can be handled in the air and stored at room temperature for months without appreciable decomposition. Preliminary ab initio calculations carried out with compound **1a** indicated that a possible reason for that high stability would be the formation of a hydrogen bond between the iminic-hydrogen and the amido carbonyl group present in the ampicillin side chain (Fig. 2).

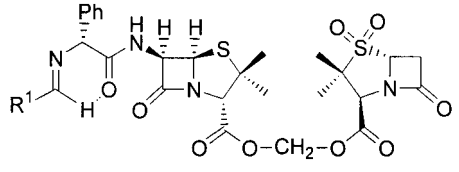


Figure 2.

Although ampicillin imines **7** were usually prepared and used in situ, they can also be isolated just by removing the DMF and the water formed during the process. An NMR-monitored experiment showed that the formation of ampicillin imine derived from benzaldehyde is complete after 2 h. Benzaldehyde was added to a solution of the sodium salt of ampicillin **6** in DMF-d_7 and the mixture was placed in an NMR tube. The ^{1}H NMR spectrum showed that after 12 min 50% of the starting material had been converted into the corresponding imine. In half an hour the conversion was 90% and, after 2 h, total consumption of the starting sodium salt **6** was observed. On the other hand, the reaction of ampicillin imines **7** with the sulbactam derivative **4a** was almost instantaneous.

Finally, sultamicillin imines were transformed in a selective and easy way into sultamicillin base **5** (sultamicillin base is usually prepared from its tosylate by treatment with iPr$_2$EtN). The imine deprotection reaction was carried out with Girard reagents.[14] These reagents are quaternary ammonium salts which bear in their structure an acetylhydrazine functionality. Since they were introduced by Girard and Sandulesco in 1936, they have been widely used in natural product chemistry, to extract carbonylic compounds from natural product mixtures.[15] The treatment of a solution of the imino derivatives **1**, in a polar protic solvent like methanol, with a Girard reagent in the presence of *p*-toluenesulfonic acid leads, after addition of a K_2HPO_4 solution (that affords a final pH between 8 and 8.5) and extractive work up, to the sultamicillin base **5** in very good yields (Scheme 1).[16] We have also run this reaction with acetylhydrazine and 2,4-dinitrophenylhydrazine and the deprotection took place as well, but it was not possible to separate the base **5** from the hydrazone formed. The Girard reagent used is an ammoniun salt so, the final hydrazone formed is retained in the aqueous layer during the extractive workup, and the base is obtained in a pure form without further purification.

Clearly, the procedure described herein to prepare sultamicillin base **5** is an adequate method to synthesize this compound, under mild conditions and in high yields. In addition, the base is usually prepared starting from its tosylate form, while this methodology allows its direct formation, avoiding the tosylate formation step.

In summary, we have disclosed the synthesis of sultamicillin imines **1**. These new compounds showed high stability, allowing the use of aqueous workup and flash chromatography techniques and facilitating its handling and purification, in deep contrast with the enamine counterparts (these enamines are currently used as *N*-protecting groups for ampicillin in sultamicillin synthesis).[7d,e] Furthermore, they are convenient starting materials for the efficient preparation of sultamicillin base by deprotection with Girard reagents.

3. Experimental

Solvents were purchased from Aldrich. Chromatographic purifications were performed on silica gel (230–400 mesh) by flash technique. Analytical TLC plates (covered with silica gel 60 F_{254}) were viewed by UV light or developed by heating after treatment with an acidic solution of Ce(IV) and Mo(VI). NMR spectra were recorded on a Bruker AC-300 or an Avance DPX-300 spectrometer. Both instruments were equipped with a QNP-probe ^{1}H, ^{13}C, ^{15}N, ^{31}P which, in the case of the DPX-300 apparatus, included a shielded Z-gradient coil. Chemical shifts are referred to TMS as internal standard. 1D (^{1}H, ^{13}C, DEPT-135) and 2D (gHMQC and gHMBC) spectra were acquired and processed using the standard software implemented in the spectrometers. Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. IR analyses were performed on a Perkin–Elmer Paragon 1000 FTIR spectrometer.

3.1. General procedure for the preparation of sultamicillin imines 1

To a stirred solution of sodium ampicillin (1.13 g, 3 mmol) in DMF (12 mL) at 0°C was added the corresponding aldehyde (3.6 mmol) and the reaction mixture was stirred at this temperature for an additional 8 h. After this period, iodomethyl 1,1-dioxopenicillanate (1.34 g, 3.6 mmol) was added and stirring continued for 2 h. The pale yellow reaction mixture was then quenched with water (25 mL) and extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with water (2×25 mL), and dried over sodium sulfate. Elimination of the solvents at reduced pressure gave a syrup that was treated with ethyl ether (50 mL). Compounds **1** precipitated from the solution and were obtained in good yields by filtration.

3.1.1. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(benzylidenaminophenylacetamido)] penicillanate (1a). The compound was prepared according to the general procedure described above using benzaldehyde (0.37 mL, 3.6 mmol) to afford 1.86 g (2.73 mmol) of **1a** as a white solid in 91% yield: mp 123–125°C; IR (KBr) 3345, 2976, 1794, 1690, 1645, 1595 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.12 (d, J=9.5 Hz, 1H), 7.80–7.84 (m, 2H), 7.28–7.47 (m, 2H), 5.84–5.95 (ABq, J=5.6 Hz, 2H), 5.77 (dd, J=9.5, 4.3 Hz, 1H), 5.55 (d, J=4.3 Hz, 1H), 5.04 (s, 1H), 4.62–4.65 (m, 1H), 4.54 (s, 1H), 4.43 (s, 1H), 3.47 (m, 2H), 1.65 (s, 3H), 1.60 (s, 3H), 1.50 (s, 3H), 1.43 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.46, 170.97, 170.58, 166.14, 165.60, 163.49, 138.37, 135.07, 131.57, 128.71, 128.58, 128.39, 128.03, 127.18, 80.44, 75.81, 69.93, 67.99, 64.30, 62.68, 62.44, 60.81, 58.57, 38.18, 31.70, 26.60, 20.02, 18.17. HRMS calcd for $C_{32}H_{34}N_4S_2O_9$ (M+1): 683.1845, found: 683.1864. LRMS (FAB): 683 (100), 595 (50), 405 (34), 228 (58), 194 (42).

3.1.2. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(2-nitrophenylmethylidenaminophenylacetamido)] penicillanate (1b). The compound was prepared according to the general procedure described above using 2-nitrophenylcarboxaldehyde (0.54 g, 3.6 mmol) to afford 1.61 g (2.22 mmol) of **1b** as a pale yellow solid in 74% yield: mp 130–132°C; IR (KBr) 3360, 2976, 1792, 1684, 1637, 1574 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 8.03–8.19 (m, 2H), 7.85 (d, J=9.9 Hz, 1H), 7.58–7.75 (m, 2H), 7.30–7.55 (m, 5H), 5.86–5.96 (ABq, J=5.6 Hz, 2H), 5.79 (dd, J=4.2 and 9.4 Hz, 1H), 5.57 (d, J=4.4 Hz, 1H), 5.16 (s, 1H), 4.62–4.65 (m, 1H), 4.52 (s, 1H), 4.43 (s, 1H), 3.47–3.50 (m, 2H), 1.63 (s, 3H), 1.61 (s, 3H), 1.51 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.31, 170.65, 170.26, 166.13, 165.64, 159.84, 148.81, 137.68, 133.34, 131.56, 129.97, 129.59, 128.89, 128.31, 127.25, 124.44, 80.48, 76.24, 69.99, 67.95, 64.39, 62.72, 62.47, 60.87, 58.53, 38.19, 31.46, 26.68, 20.02, 18.19. HRMS calcd for $C_{32}H_{33}N_5S_2O_{11}$ (M+1): 728.7747, found: 728.7741. LRMS (FAB): 728 (100), 595 (44), 239 (63).

3.1.3. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(3-nitrophenylmethylidenaminophenylacetamido)] penicillanate (1c). The compound was prepared according to the general procedure described above using 3-nitrophenylcarboxaldehyde (0.54 g, 3.6 mmol) to afford 1.48 g (2.04 mmol) of **1c**

as a pale yellow solid in 68% yield: mp 113–115°C; IR (KBr) 3341, 2978, 1793, 1690, 1648, 1617 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.84–8.87 (m, 1H), 8.38 (s, 1H), 8.30–8.38 (m, 1H),8.18 (d, J=9.8 Hz, 1H), 7.96–8.01 (m, 1H), 7.60–7.70 (m, 1H), 7.31–7.48 (m, 5H), 5.86–5.97 (ABq, J=5.6 Hz, 2H), 5.82 (dd, J=4.4 and 9.8 Hz, 1H),5.59 (d, J=4.4 Hz, 1H), 5.12 (s, 1H), 4.63–4.72 (m, 1H), 4.59 (s, 1H), 4.44 (s, 1H), 3.47–3.50 (m, 2H), 1.76 (s, 3H), 1.61 (s, 3H), 1.57 (s, 3H), 1.45 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.22, 170.62, 170.09, 166.05, 165.62, 160.98, 148.52, 137.82, 136.65, 134.82, 129.81, 128.90, 128.32, 127.10, 125.78, 121.97, 80.51, 75.73, 69.97, 67.95, 64.64, 62.69, 62.43, 60.85, 58.53, 38.16, 32.13, 26.39, 30.00, 18.16. HRMS calcd for $C_{32}H_{33}N_5S_2O_{11}$ (M+1): 728.7747, found: 728.7739. LRMS (FAB): 728 (100), 595 (65), 239 (72).

3.1.4. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(2-chlorophenylmethylidenaminophenylacetamido)] penicillanate (1d). The compound was prepared according to the general procedure described above using 2-chlorophenylcarboxaldehyde (0.41 mL, 3.6 mmol) to afford 1.53 g (2.13 mmol) of **1d** as a pale yellow solid in 71% yield: mp 122–124°C; IR (KBr) 3349, 2977, 1790, 1694, 1637, 1592 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.15 (d, J=7.5 Hz, 1H), 7.95 (d, J=9.5 Hz, 1H), 7.27–7.44 (m, 8H), 5.83–5.92 (ABq, J=5.6 Hz, 2H), 5.74 (dd, J=9.5 and 4.2 Hz, 1H), 5.53 (d, J=4.2 Hz, 1H), 5.09 (s, 1H), 4.62 (m, 1H), 4.51 (s, 1H), 4.41 (s, 1H), 3.45 (m, 2H), 1.61 (s, 3H), 1.58 (s, 3H), 1.48 (s, 3H), 1.41 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.39, 170.62, 166.11, 165.63, 160.36, 138.20, 135.72, 132.44, 132.10, 129.99, 128.79, 128.21, 128.16, 127.13, 126.84, 80.47, 76.33, 69.96, 68.01, 64.34, 62.73, 62.46, 60.86, 58.58, 38.19, 31.67, 26.63, 20.03, 18.20. HRMS calcd for $C_{32}H_{33}ClN_4S_2O_9$ (M+1): 717.2142, found: 717.2132. LRMS (FAB): 717 (100), 595 (87), 228 (42).

3.1.5. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(4-chlorophenylmethylidenaminophenylacetamido)] penicillanate (1e). The compound was prepared according to the general procedure described above using 4-chlorophenylcarboxaldehyde (0.51 g, 3.6 mmol) to afford 1.81 g (2.52 mmol) of **1e** as a pale yellow solid in 84% yield: mp 110–112°C; IR (KBr) 3348, 2976, 1794, 1686, 1647, 1596 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.99 (d, J=9.6 Hz, 1H), 7.70 (d, J=8.3 Hz, 2H), 7.20–7.40 (m, 7H), 5.79–5.88 (ABq, J=5.7 Hz, 2H), 5.71 (dd, J=9.6 and 4.4 Hz, 1H), 5.50 (d, J=4.4 Hz, 1H), 4.99 (s, 1H), 4.58–4.60 (m, 1H), 4.50 (s, 1H), 4.38 (s, 1H), 3.35–3.39 (m, 2H), 1.59 (s, 3H), 1.53 (s, 3H), 1.45 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.22, 170.64, 170.42, 165.93, 165.49, 162.03, 138.12, 137.29, 133.41, 129.42, 128.73, 128.58, 127.93, 127.02, 80.31, 75.65, 69.71, 67.80, 64.18, 62.44, 62.22, 60.68, 58.36, 37.96, 31.67, 26.40, 20.74, 17.92. HRMS calcd for $C_{32}H_{33}ClN_4S_2O_9$ (M+1): 717.2142, found: 717.2136. LRMS (FAB): 717 (100), 595 (67), 228 (65).

3.1.6. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(2-furylmethylidenaminophenylacetamido)] penicillanate (1f). The compound was prepared according to the general procedure described above using 2-furaldehyde (0.30 mL, 3.6 mmol) to afford 1.79 g (2.67 mmol) of **1f** as a pale yellow solid in 89% yield: mp 118–120°C; IR (KBr) 3348, 2977, 1790, 1688, 1646, 1506 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (s, 1H), 8.04 (d, J=10.1 Hz, 1H), 7.57-7.60 (m, 1H), 7.25–7.45 (m, 5H), 6.87–6.89 (m, 1H), 6.51–6.54 (m, 1H), 5.85–5.96 (ABq, J=5.7 Hz, 2H), 5.74 (dd, J=9.3 and 4.4 Hz, 1H), 5.55 (d, J=4.4 Hz, 1H), 4.98 (s, 1H), 4.62–4.65 (m, 1H), 4.52 (s, 1H), 4.43 (s, 1H), 3.36–3.50 (m, 2H), 1.65 (s, 3H), 1.61 (s, 3H), 1.50 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.35, 170.83, 170.62, 166.16, 165.62, 151.66, 151.00, 145.47, 138.14, 128.76, 128.09, 127.40, 115.52, 111.94, 80.46, 75.93, 69.95, 67.98, 64.23, 62.71, 62.45, 60.84, 58.69, 38.16, 31.54, 26.59, 20.00, 18.17. HRMS calcd for $C_{30}H_{32}N_4S_2O_{10}$ (M^+): 672.7303, found: 672.7296. LRMS (FAB): 672 (100), 595 (46), 184 (77).

3.1.7. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(3-pyridylmethylidenaminophenylacetamido)] penicillanate (1g). The compound was prepared according to the general procedure described above using 3-pyridylcarboxaldehyde (0.34 mL, 3.6 mmol) to afford 1.93 g (2.82 mmol) of **1g** as a pale yellow solid in 94% yield: mp 118–120°C; IR (KBr) 3350, 2978, 1793, 1690, 1647, 1590 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.68 (d, J=3.5 Hz, 1H), 8.32 (s, 1H), 8.12 (d, J=7.9 Hz, 1H), 7.96 (d, J=9.6 Hz, 1H), 7.27–7.51 (m, 6H), 5.83–5.92 (ABq, J=5.6 Hz, 2H), 5.75 (dd, J=9.6 and 4.3 Hz, 1H), 5.53 (d, J=4.3 Hz, 1H), 5.06 (s, 1H), 4.60–4.62 (m, 1H), 4.52 (s, 1H), 4.40 (s, 1H), 3.37–3.51 (m, 2H), 1.63 (s, 3H), 1.57 (s, 3H), 1.48 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.28, 170.56, 170.40, 166.06, 165.59, 160.97, 152.14, 150.03, 137.86, 134.95, 130.61, 128.83, 128.25, 127.18, 123.61, 80.44, 76.11, 69.93, 67.91, 64.42, 62.68, 62.42, 60.81, 58.46, 38.16, 31.69, 26.58, 19.99, 18.16. HRMS calcd for $C_{31}H_{33}N_5S_2O_9$ (M+1): 684.7568, found: 684.7556. LRMS (FAB): 684 (100), 595 (79), 195 (62).

3.1.8. 1,1-Dioxopenicillanoyloxymethyl 6-[D-α-(*t*-butylmethylidenaminophenylacetamido)] penicillanate (1h). The compound was prepared according to the general procedure described above using pivalaldehyde (0.33 mL, 3.6 mmol) to afford 1.61 g (2.43 mmol) of **1h** as a pale yellow solid in 81% yield: mp 187–189°C; IR (KBr) 3348, 2970, 1794, 1687, 1507, cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J=9.8 Hz, 1H), 7.61 (s, 1H), 7.27–7.49 (m, 5H), 5.85–5.96 (ABq, J=5.7 Hz, 2H), 5.78 (dd, J=10.1 and 4.4 Hz, 1H), 5.55 (d, J=4.4 Hz, 1H), 4.77 (s, 1H), 4.63–4.67 (m, 1H), 4.53 (s, 1H), 4.43 (s, 1H), 3.47–3.50 (m, 2H), 1.69 (s, 3H), 1.61 (s, 3H), 1.53 (s, 3H), 1.44 (s,3H), 1.12 (S, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.24, 173.70, 171.23, 170.61, 166.21, 165.66, 138.61, 128.61, 127.84, 126.88, 80.50, 75.58, 70.02, 68.18, 64.50, 62.77, 62.52, 60.90, 58.37, 38.24, 36.85, 31.83, 26.74, 26.55, 20.08, 18.25. HRMS calcd for $C_{30}H_{38}N_4S_2O_9$ (M+1): 662.7788, found: 662.7776. LRMS (FAB): 663 (100), 595 (59), 174 (46).

3.2. General procedure for the synthesis of sultamicillin base 5

To a stirred solution of *p*-toluenesulfonic acid (0.42 g, 2.2 mmol) and Girard-P (0.42 g, 2.2 mmol) in methanol (20 mL), was added at room temperature 1.36 g (2 mmol) of the sultamicillin imine **1a**. The reaction mixture was

stirred for 30 min. After this period, methanol was removed under reduced pressure and 30 mL of methylene chloride was added. The solution was then quenched with 30 mL of 1 M K_2HPO_4 solution and extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with brine (2×25 mL), dried over sodium sulfate and concentrated in vacuo. The resultant crude reaction mixture was diluted with isopropanol (20 mL) and stirred for 2 h. Sultamicillin base **5** was separated by filtration as a white solid in 80% yield. The ^{1}H NMR of the product obtained was identical to the one showed by the pure material. IR (KBr, cm^{-1})=3400, 3300, 1800, 1785, 1755, 1680, 1510. ^{1}H NMR (200 MHz, $CDCl_3$) (δ, ppm): 1.43 (s, 3H); 1.51 (s, 3H); 1.60 (s, 3H); 1.66 (s, 3H); 1.94 (s ancho, 2H); 3.47 (m, 2H); 4.43 (s, 1H); 4.48 (s, 1H); 4.57 (s, 1H); 4.63(dd, J=3.7 and 2.6 Hz, 1H); 5.52 (d, J=4.4 Hz, 1H); 5.68 (dd, J=9.5 and 4, 4 Hz, 1H); 5.85–5.95 (Abq, J=5.5 Hz, 2H); 7.33–7.39 (m, 5H arom.); 8.11 (d, J=9.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) (δ, ppm): 18.21 (q); 20.05 (q); 26.79 (q); 31.01 (q); 38.21 (t); 58.31 (d); 59.54 (d); 60.84 (d); 62.47 (s); 62.71 (d); 64.42 (s); 67.95 (d); 70.08 (d); 80.47 (t); 126.85 (2d); 128.19 (d); 128.90 (2d); 139.95 (s); 165.63 (s); 166.24 (s); 170.56 (s); 172.65 (s); 173.80 (s).

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