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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF TRINEMS BEARING NITROGEN DERIVATIVES AT C(4)

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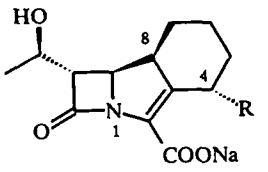
Optimization of the antibacterial activity of 4-amino trinem, obtained through chemical modification of the basicity of the amino group at position 4, has led to the identification of a very interesting compound characterized by a broad spectrum of activity including *Pseudomonas aeruginosa*.

β-Lactams [1-3] constitute a very important class of antibacterial agents which encompass penicillin, cephalosporins, monobactams, penems, carbapenems, and more recently trinems [4-11].



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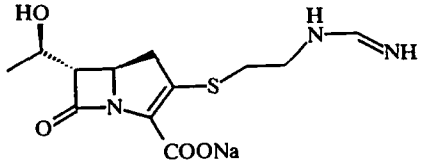


2
Imipenem

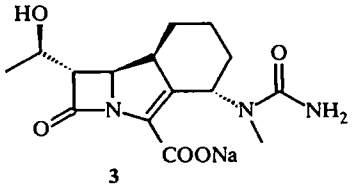
The general trend presented by 4-substituted trinems **1** is characterized by potency, broad spectrum of activity, remarkable resistance to β-lactamases, and stability to mammalian renal peptidases (DHP-1).

SAR studies [12, 13] have shown that the absolute stereochemistry at C(4) and C(8) of 4-substituted trinems is very important in modulating the antibacterial activity and other biological properties; in particular, (4*S*,8*S*) were recognized as the most effective absolute configurations.

Because of the well known epidemiological importance of *Pseudomonas aeruginosa* both in the hospital and community, the identification of a broad spectrum antibacterial compound covering this opportunistic pathogen would represent a very attractive area of research for many pharmaceutical companies involved in the antibacterial field.

Among other classes, 4-amino trinem derivatives have shown an interesting antimicrobial activity over a broad range of bacteria [14]; in particular, the activity against *P. aeruginosa* was comparable to imipenem (**2**), one of the best anti-infective agents currently in use. We thought that a compound encountering the aforementioned antibacterial profile would be obtained through a further derivatization of such a class.

As part of our research activity we focused our attention on the synthesis of a small set of trinem derivatives **3-6**, characterized by the presence of a nitrogen at C(4), whose basicity was modulated by the introduction of an hydrophilic group.



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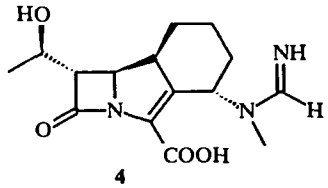

4

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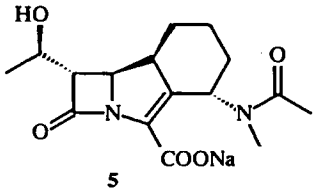

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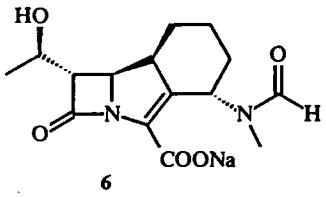
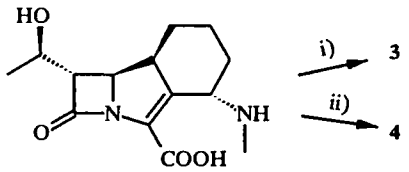


6

SYNTHESIS

Trinems **3** [15, 16] and **4** [17] were obtained starting from the common and already described amino acid **7** [14], as outlined in Scheme 1.

Scheme 1

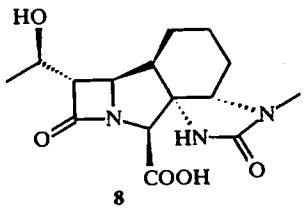


7

i) TMSNCO; ii) benzyloxyformimidate hydrochloride, phosphate buffer pH 8

The reduced solubility of the amino acid **7** in many organic solvents forced us to perform the final conversion to 4-N-methylureido trinem **3** in water. Compound **7** was dissolved in water and treated with an excess of trimethylsilylisocyanate (TMSNCO) to give the crude 4-N-methylureido trinem **3**, which was purified by reverse phase chromatography (RP-18). This compound quite rapidly underwent an intramolecular Michael addition giving the tetracyclic saturated compound **8**. This irreversible reaction was followed by ^1H NMR spectroscopy in D_2O at 22 °C, obtaining an almost complete conversion to **8** in 24 h. The absolute stereochemistry of compound **8** was confirmed by NOE experiments and a more detailed analysis of such experiments will be reported elsewhere.

A pure sample of compound **8** when tested *in vitro* was proved to be completely inactive from the microbiological point of view.



8

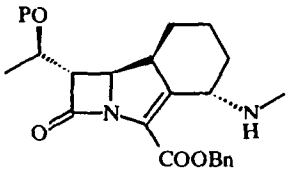
A freshly purified sample of compound **3** was assayed against several bacterial strains (see Table 1), showing a significant antimicrobial activity including *P. aeruginosa*.

Compound **4** was prepared reacting benzyloxyformimidate hydrochloride with the amino acid **7** in a buffered solution at pH 8. The compound was purified by reverse phase chromatography and isolated after freeze drying and its microbiological profile evaluated.

The availability of **7** prompted us to attempt the synthesis of the other two 4-amino trinem derivatives **5** and **6** in the aqueous phase using the corresponding acylating agents, acetic anhydride and acetic formic anhydride (AFA) [12]; however, as expected, the reactivity of these systems in water was very poor giving the desired compounds in

low yields. Therefore, we were forced to introduce the formyl and the acetyl moiety using a more suitable precursor of trinem **7**, i.e,. the amino ester **9**.

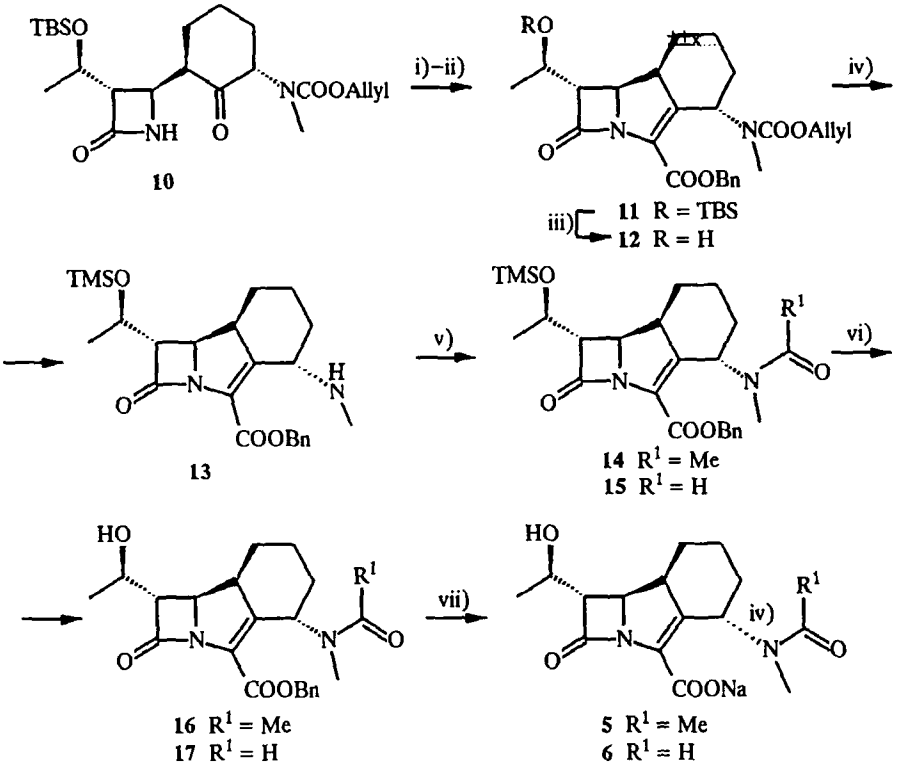
The latter was obtained starting from the azetidinone **10** and using protection of the N(4) and the carboxy group at C(2).



9, P = protecting group or H

We chose as the most appropriate combination allyloxycarbonyl and benzyl ester as protecting groups for the nitrogen and the carboxyl moiety respectively. In Scheme 2 is depicted the synthetic route followed to obtain compounds **5** and **6**.

Scheme 2



i) ClCOCOOBn, pyridine, DCM; ii) $P(OEt)_3$, toluene, 110 °C; iii) tetrabutylammonium fluoride (TBAF), AcOH, THF; iv) Me_2NSiMe_3, $Pd(Ph_3P)_4$; v) Ac_2O or AFA, TEA; vi) TBAF, AcOH, THF; vii) H_2, Pd/C

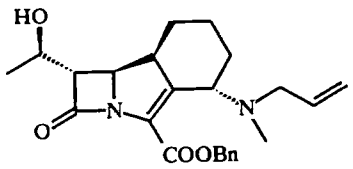
Intermediate **10**, whose synthesis is described in a forthcoming communication submitted for publication in *Tetrahedron*, was converted into the trinem **11** using the well known two-step cyclization procedure [19-21]. The

TABLE 1. Minimum Inhibitory Concentrations (MIC) Determined in Mueller Hinton Broth; Anaerobes Schadler Broth Inoculum = 5×10^5 CFU/ml

	S. aur. 663	*S. aur.* 853	*S. pne.* 3512	*E. coli* 1850	*P. aer.* 1911	*B. frag.* 2017	*C. per.* 615
Imipenem	0.06	0.12	0.01	1.0	4.0	0.12	0.03
3	0.12	0.12	0.12	0.12	8.0	0.12	0.12
4	0.12	0.12	0.06	0.5	4.0	0.12	0.03
5	0.25	0.25	0.25	0.25	>32	0.25	0.25
6	0.5	1.0	0.01	0.25	32	0.5	0.03
7	0.25	0.5	1.0	2.0	4.0	2.0	0.5

S. aur. 663 = *Staphylococcus aureus* 663E;
S. aur. 853 = *Staphylococcus aureus* 853E β-lactamases producing strains;
S. pne. 3512 = *Streptococcus pneumoniae* 3512;
E. coli 1850 = *Escherichia coli* 1850E;
P. aer. 1911 = *Pseudomonas aeruginosa* 1911 wild type ;
B. frag. 2017 = *Bacteroides fragilis* 2017E;
C. per. 615 = *Clostridium perfringens* 615E.

t-butyldimethylsilyl (TBS) protecting group was removed from compound **11** to give **12** in satisfactory yield, but unexpectedly the subsequent deprotection of the 4-methylamino functionality using Pd^0 and Na 2-ethylhexanoate as allyl trapping agent gave considerable amounts of the corresponding N-allylated derivative **18**.



18

The suppression of this side reaction was achieved by using the more nucleophilic Me_2NTMS with simultaneous silylation of the hydroxyethyl side chain to give **13**. Compounds **14** and **15** were obtained by reacting **13** with acetic anhydride and acetic formic anhydride respectively. Both intermediates were smoothly converted to the corresponding trinems **5** and **6** in two steps, according to the well known procedure [22].

The antibacterial activity of the final compounds was evaluated against a series of Gram-positive and Gram-negative aerobic and anaerobic bacterial strains. In Table 1 is reported a selection of the *in vitro* antibacterial activity (MIC μg/ml) of trinems **3-7** in comparison with imipenem as determined by the microtiter broth dilution test (MIC values).

Masking of basic functional groups by N-acetylation (**5**) and N-formylation (**6**) considerably reduced the anti-pseudomonal activity of trinems, as previously suggested [23], while the original activity showed by trinem **7** was retained by compounds **3** and in particular by **4**, which gave a broader spectrum of activity.

Furthermore these compounds showed good stability to renal human dehydropeptidase and to β-lactamases (compare activities against *S. aureus* 853 and 663).

In conclusion modulation of the basicity of **7** by introduction of a carbonyl group has generally resulted in less potent derivatives in particular against *P. aeruginosa*. However, in the case of trinem **3** we have observed an improved

spectrum of antibacterial activity but a slightly reduced antipseudomonal activity compared to trinem **7**. Only with the introduction of the formamidino group, which retains a certain degree of basic character, did we observe both a comparable antipseudomonal activity and an enhancement in the antibacterial spectrum with respect to trinem **7**. The interesting results observed with the 4-formamidino trinem derivative (**4**) prompted us to consider it as a good prototype [24] and to further our investigation into the evaluation of close analogues.

EXPERIMENTAL

The IR spectra were recorded with IISF48 Bruker spectrophotometer. Mass spectra (MS) were recorded with a VG quattro FISONS using FAB^+ ionization technique. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a Varian XL-400 MHz and VXR 300 MHz and measured in D_2O or $CDCl_3$. Chemical shift are given in ppm scale.

Benzyl (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-(tert-butyldimethylsiloxy)ethyl]-11-oxo-4(N-allyloxycarbonyl)methylaminotricyclo[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (11). To a stirred solution of **10** (1 g) in dichloromethane, pyridine (0.3 ml) and benzyloxalyl chloride (0.3 ml) were added at 0°C. After 30 min the solution was diluted with ethyl acetate and washed with a saturated solution of ammonium chloride, sodium hydrogencarbonate, and brine. The organic solution was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The oily residue was dissolved in dry toluene and triethoxyphosphine (1 ml) was added, and the resulting solution was heated at 110°C for 6 h. The cooled organic solution was concentrated under reduced pressure and the oily residue was purified by flash chromatography to give **11** (0.97 g) in 75% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.45-7.27 (5H, m); 5.96-5.30 (1H, m); 5.36 (1H, t); 5.32-5.14 (4H, m); 4.52 (2H, m); 4.20 (1H, m); 4.18 (1H, dd); 3.22-3.12 (2H, dd+m); 2.98 (3H, s); 2.22-2.12 (1H, m); 1.92-1.82 (1H, m); 1.75-1.54 (3H, m); 1.48-1.30 (1H, m); 1.22 (3H, d); 0.86 (9H, s); 0.065 (6H, s). IR ($CDCl_3$, ν, cm^{-1}) 1772, 1715, 1691. MS (FAB^+) 569 (MH^+). Found, %: C 68.90; H 8.13; N 5.06. $C_{31}H_{44}N_2O_6Si$. Calculated, %: C 68.84; H 8.21; N 5.18.

Benzyl (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-hydroxyethyl]-11-oxo-4-(N-allyloxycarbonyl)methylaminotricyclo[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (12). Intermediate **11** (0.9 g) was dissolved in tetrahydrofuran, then acetic acid (1.5 ml) and tetrabutylammonium fluoride trihydrate (7 g) were added. After 8 h the solution was diluted with ethyl acetate and washed with a saturated solution of sodium hydrogen carbonate and brine. The organic solution was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The oily residue was purified by flash chromatography to give **12** (0.5 g) in 70% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.44-7.28 (5H, m); 5.86 (2H, m); 5.35 (1H, t); 5.34-5.15 (4H, m); 4.51 (2H, m); 4.20 (3H, dd+m); 3.25-3.22 (2H, dd+m); 2.96 (3H, s); 2.15 (2H, m); 2.00-1.31 (6H, d+m). IR ($CDCl_3$, ν, cm^{-1}) 1774, 1720, 1691. MS (FAB^+) 455 (MH^+). Found, %: C 66.10; H 6.91; N 6.22. $C_{25}H_{30}N_2O_6$. Calculated, %: C 66.04; H 6.66; N 6.17.

Benzyl (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-(trimethylsilyloxy)ethyl]-11-oxo-4-(N-acetyl)methylaminotricyclo[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (14). Intermediate **12** (0.22 g) was dissolved in dry dichloromethane, then trimethyl(dimethylamino)silane and tetrakis(tri-phenylphosphine)palladium were added. After 1 h, the volatile organic meterial was removed under reduced pressure to give the crude material **13** which was immediately used for the next reaction.

The material was dissolved in dry dichloromethane, then triethylamine followed by acetic anhydride were added. After 20 min, the solution was diluted with ethyl acetate and washed with a saturated solution of ammonium chloride and brine. The organic solution was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The oily residue was purified by flash chromatography to give **14** (0.14 g) in 70% yield.

Compound **13**: ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.45 (2H, d); 7.35 (2H, t); 7.30 (1H, m); 5.35 (1H, d); 5.21 (1H, d); 4.40 (1H, t); 4.20-4.10 (2H, m+dd); 3.20 (2H, m+dd); 2.29 (3H, s); 1.90-1.77 (3H, m); 1.65-1.50 (2H, m); 1.35 (2H, m); 1.24 (3H, d); 0.10 (9H, s). IR ($CDCl_3$, ν, cm^{-1}); 1769, 1720. MS (FAB^+) 443 (MH^+).

Compound **14**: ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.44-7.34 (5H, m); 5.26-5.20 (3H, m+dd); 4.20 (1H, m); 4.12 (1H, dd); 3.21 (1H, dd); 3.02 (1H, m); 2.83 (3H, s); 2.32 (1H, m); 2.06 (3H, s); 1.85-1.33 (5H, m); 1.20 (3H, d); 0.07 (9H, s). IR ($CDCl_3$, ν, cm^{-1}): 1769, 1720. MS (FAB^+) 485 (MH^+). Found, %: C 68.50; H 8.03; N 6.40. $C_{26}H_{36}N_2O_6$. Caculated, %: C 68.38; H 7.95; N 6.14.

Benzyl (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-(trimethylsiloxy)ethyl]-11-oxo-4-(N-formyl)methylaminotricyclo-[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (15). The same procedure followed for **14** was repeated to give **15** (0.115 g) in 60% yield when AFA was used instead of acetic anhydride. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.23 (1H, s); 7.43-7.35 (5H, m); 5.27-5.23 (3H, m+dd); 4.22 (1H, m); 4.17 (1H, dd); 3.22 (1H, dd); 3.05 (1H, m); 2.86 (3H, s); 2.30 (1H,

m); 1.90-1.34 (5H, m); 1.22 (3H, d); 0.08 (9H, s). IR ($CDCl_3$, ν, cm^{-1}): 1775, 1721. MS (FAB^+) 471 (MH^+). Found, %: C 67.80; H 7.50; N 6.43. $C_{25}H_{34}N_2O_5Si$. Calculated, %: C 67.83; H 7.75; N 6.33.

Benzyl (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-hydroxyethyl]-11-oxo-4-(N-acetyl)methylaminotricyclo[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (16). Intermediate **14** (0.14 g) was dissolved in tetrahydrofuran, then acetic acid (0.025 ml) and tetrabutylammonium fluoride trihydrate (0.12 g) were added. After 2 h the solution was diluted with ethyl acetate and washed with a saturated solution of sodium hydrogen carbonate and brine. The organic solution was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The oily residue was purified by flash chromatography to give **16** (0.095 g) in 80% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.48-7.33 (5H, m); 5.33-5.21 (3H, d+d+m); 4.35-4.18 (2H, dd+m); 3.29 (1H, dd); 3.02 (1H, m); 2.82 (3H, s); 2.33 (1H, m); 2.09 (3H, s); 1.81 (1H, d); 1.42 (5H, m); 1.32 (3H, d). IR ($CDCl_3$, ν, cm^{-1}): 1779, 1719, 1660. MS (FAB^+) 413 (MH^+). Found, %: C 66.80; H 6.81; N 6.94. $C_{23}H_{28}N_2O_5$. Calculated, %: C 66.95; H 6.85; N 6.80.

Benzyl (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-hydroxyethyl]-11-oxo-4-(N-formyl)methylaminotricyclo[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (17). Using the same procedure followed for obtaining **16**, the compound **15** (0.115 g) was converted to **17** (0.07 g) in 72% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.22 (1H, s); 7.46-7.32 (5H, m); 5.35-5.22 (3H, d+d+m); 4.30-4.20 (2H, dd+m); 3.27 (1H, dd); 3.10 (1H, m); 2.85 (3H, s); 2.36 (1H, m); 1.79 (1H, d); 1.40 (5H, m); 1.32 (3H, d). IR ($CDCl_3$, ν, cm^{-1}); 1778, 1720, 1660. MS (FAB^+) 399 (MH^+). Found, %: C 66.40; H 7.00; N 6.91. $C_{22}H_{26}N_2O_5$. Calculated, %: C 66.30; H 6.58; N 7.03.

Sodium (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-hydroxyethyl]-11-oxo-4-(N-acetyl)methylaminotricyclo[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (5). Intermediate **16** (0.09 g) was dissolved in tetrahydrofuran and water (3:1), then palladium on charcoal (10%) was added and the resulting mixture was hydrogenated monitoring the progress of the reaction by TLC. When the reaction was completed, sodium hydrogen carbonate (0.021 g) was added and the organic solvent removed under reduced pressure. The resulting aqueous phase was freeze dried to give **5** (0.07 g) in 95% yield. ^{1}H-NMR (D_2O, 400 MHz): 4.17 (2H, m); 3.40 (1H, dd); 3.27 (1H, m); 3.00-2.84 (3H, m); 2.18-2.00 (3H, m); 1.88 (1H, m); 1.78-1.42 (3H, m); 1.39-1.10 (5H, m); 0.82-0.74 (2H, m). IR ($CDCl_3$, ν, cm^{-1}) 1772, 1720. MS (FAB^+) 345 ($MH + Na^+$), 323 (MH^+). Found, %: C 56.00; H 6.09; N 8.03. $C_{16}H_{21}N_2NaO_5$. Calculated, %: C 55.79; H 6.15; N 8.14.

Sodium (4*S*,8*S*,9*R*,10*S*,12*R*)-1-Aza-10-[1-hydroxyethyl]-11-oxo-4-(N-formyl)methylaminotricyclo[7,2,0,$0^{3,8}$]undec-2-ene-2-carboxylate (6). Using the same procedure followed for obtaining **5**, the compound **17** (0.07 g) was converted to **6** (0.055 g) in 97% yield. ^{1}H-NMR (D_2O, 400 MHz): 8.00 (1H, s); 4.98 (1H, t); 4.05 (1H, m); 4.03 (1H, dd); 3.29 (1H, dd); 2.96-2.86 (1H, m); 2.68 (3H, s); 2.19 (1H, m); 1.75 (1H, m); 1.70-1.2 (5H, m); 1.09 (3H, d). IR ($CDCl_3$, ν, cm^{-1}) 1777, 1718. MS (FAB^+) 331 ($MH + Na^+$), 309 (MH^+). Found, %: C 54.40; H 5.50; N 8.61. $C_{15}H_{19}N_2NaO_5$. Calculated, %: C 54.52; H 5.80; N 8.49.

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REFERENCES

1. Z. Zhang, Zhongguo Linchuang Yaolixue Zazhi, **13**, No. 3, 163 (1997).
2. J. Parrick and L. K. Mehta, Progr. Heterocycl. Chem., **8**, 66 (1996).
3. K. Bush, Antibiot. Chemother. (7th Ed.), F. O'Grady (ed.): Churchill Livingstone, Edinburgh, UK (1997), p. 306.
4. B. Tamburini, A. Perboni, T. Rossi, D. Donati, D. Andreotti, G. Gaviraghi, R. Carlesso, and C. Bismara, Eur. Pat. Appl. EP0416953 A2; Chem. Abstr., **116**, 235337 (1992).
5. R. Di Fabio, D. Andreotti, S. Biondi, G. Gaviraghi, and T. Rossi, Bioorg. Med. Chem. Lett., **6**, 2025 (1996).
6. D. Andreotti, S. Biondi, R. Di Fabio, D. Donati, E. Piga, and T. Rossi, Bioorg. Med. Chem. Lett., **6**, 2019 (1996).
7. P. M. Jackson, S. M. Roberts, S. Davalli, D. Donati, C. Marchioro, A. Perboni, S. Proviera, and T. Rossi, J. Chem. Soc., Perkın Trans. I, No. 16, 2029 (1996).
8. R. Camerini, M. Panunzio, G. Bonanomi, D. Donati, and A. Perboni, Tetrahedron Lett., **37**, 2467 (1996).
9. G. Gaviraghi, Eur. J. Med. Chem. (Suppl., Proceedings of the 13th International Symposium on Medicinal Chemistry, 1994), **30**, 67 (1995).
10. S. Hanessian, A. Griffin, and M. J. Rozema, Bioorg. Med. Chem. Lett., **7**, 1857 (1997).

11. S. Hanessian, M. Rozema, G. B. Reddy, and J. F. Braganza, Bioorg. Med. Chem. Lett., **5**, 2535 (1995).
12. S. Biondi, Anti-Infectives Recent Advances in Chemistry and Structure-Activity Relationships. P. H. Bentley and P. J. O'Hanlon (eds.), The Royal Society of Chemistry, Cambridge (1997), p. 86.
13. D. Andreotti, T. Rossi, G. Gaviraghi, D. Donati, C. Marchioro, E. Di Modugno, and A. Perboni, Bioorg. Med. Chem. Lett., **6**, 491 (1996).
14. M. E. Tranquillini, G. L. Araldi, D. Donati, G. Pentassuglia, A. Pezzoli, and A. Ursini, Bioorg. Med. Chem. Lett., **6**, 1683 (1996).
15. A. Perboni, G. Pentassuglia, D. Andreotti, and J. A. Winders, PCT Int. Appl., WO 9513278; Chem. Abstr., **123**, 198515 (1995).
16. S. Gehanne, E. Piga, D. Andreotti, S. Biondi, and D. Pizzi, Bioorg. Med. Chem. Lett., **6**, 2791 (1996).
17. A. Perboni, D. Donati, and G. Tarzia, Eur. Pat. Appl., EP 502468 A1; Chem. Abstr., **118**, 80719 (1993).
18. L. I. Krimen, Org. Syn., **50**, 1 (1970).
19. E. Perrone, M. Alpegiani, A. Bedeschi, F. Giudici, and G. Franceschi, Tetrahedron Lett., **25**, 2399 (1984).
20. C. Battistini, C. Scarafile, M. Foglio, and G. Franceschi, Tetrahedron Lett., **25**, 2395 (1984).
21. A. Yoshida, T. Hayashi, N. Takeda, S. Oida, and E. Ohki, Chem. Pharm. Bull., **31**, 768 (1983).
22. P. D. Jeffrey and S. W. McCombie, J. Org. Chem., **47**, 587 (1982).
23. Y. Sumita, H. Yamaga, and M. Sunagawa, J. Antibiot., **49**, 89 (1995).
24. E. Modugno, R. Broggio, I. Erbetti, and J. Lowther, Antimicrob. Agents Chemother., **41**, 2742 (1997).